

ATLAS OF INHERITED METABOLIC DISEASES

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THIRD EDITION

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Contents

Contributor	ix
Foreword	xi
Preface	xii

PART 1 ORGANIC ACIDEMIAS

1	Introduction	3
2	Propionic acidemia	8
3	Methylmalonic acidemia	19
4	Methylmalonic aciduria and homocystinuria (cobalamin C and D disease)	33
5	Multiple carboxylase deficiency/holocarboxylase synthetase deficiency	40
6	Multiple carboxylase deficiency/biotinidase deficiency	47
7	Isovaleric acidemia	57
8	Glutaric aciduria (type I)	64
9	3-Methylcrotonyl CoA carboxylase deficiency/3-methylcrotonyl glycinuria	74
10	D-2-Hydroxyglutaric aciduria	79
11	L-2-Hydroxyglutaric aciduria	85
12	4-Hydroxybutyric aciduria	89
13	Mitochondrial acetoacetyl-CoA thiolase (3-oxothiolase) deficiency	95

PART 2 DISORDERS OF AMINO ACID METABOLISM

14	Alkaptonuria	105
15	Phenylketonuria	112
16	Hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin	123
17	Biogenic amines	136
18	Homocystinuria	144
19	Maple syrup urine disease (branched-chain oxoaciduria)	152
20	Oculocutaneous tyrosinemia/tyrosine aminotransferase deficiency	164
21	Hepatorenal tyrosinemia/fumarylacetoacetate hydrolase deficiency	171
22	Nonketotic hyperglycinemia	180

PART 3 HYPERAMMONEMIA AND DISORDERS OF THE UREA CYCLE

23	Introduction to hyperammonemia and disorders of the urea cycle	191
24	Ornithine transcarbamylase deficiency	197
25	Carbamylphosphate synthetase deficiency	205
26	Citrullinemia	210
27	Argininosuccinic aciduria	216
28	Argininemia	223
29	Hyperornithinemia, hyperammonemia, homocitrullinuria syndrome	229
30	Lysinuric protein intolerance	235
31	Glutamine synthetase deficiency	241

PART 4 DISORDERS OF FATTY ACID OXIDATION

32	Introduction to disorders of fatty acid oxidation	247
33	Carnitine transporter deficiency	253
34	Carnitine-acylcarnitine translocase deficiency	260
35	Carnitine palmitoyl transferase I deficiency	267
36	Carnitine palmitoyl transferase II deficiency, lethal neonatal	273
37	Carnitine palmitoyl transferase II deficiency, late onset	277
38	Medium chain acyl CoA dehydrogenase deficiency	281
39	Very long chain acyl CoA dehydrogenase deficiency	289
40	Long chain L-3-hydroxyacyl CoA dehydrogenase – (trifunctional protein deficiency)	295
41	Short-chain acyl CoA dehydrogenase deficiency	302
42	3-HydroxyacylCoA dehydrogenase (short-chain 3-hydroxyacylCoA dehydrogenase) deficiency	309
43	Short/branched chain acyl-CoA dehydrogenase (2-methylbutyrylCoA dehydrogenase) deficiency	312
44	Multiple acyl CoA dehydrogenase deficiency/glutaric aciduria type II/ethylmalonic-adipic aciduria	316
45	3-Hydroxy-3-methylglutarylCoA lyase deficiency	325

PART 5 THE LACTIC ACIDEMIAS AND MITOCHONDRIAL DISEASE

46	Introduction to the lactic acidemias	337
47	Pyruvate carboxylase deficiency	347
48	Fructose-1,6-diphosphatase deficiency	354
49	Deficiency of the pyruvate dehydrogenase complex	359
50	Lactic acidemia and defective activity of pyruvate, 2-oxoglutarate, and branched chain oxoacid dehydrogenases	368
51	Mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS)	374
52	Myoclonic epilepsy and ragged red fiber (MERRF) disease	382
53	Neurodegeneration, ataxia, and retinitis pigmentosa (NARP)	388
54	Kearns-Sayre syndrome	393
55	Pearson syndrome	398
56	The mitochondrial DNA depletion syndromes: mitochondrial DNA polymerase deficiency	404

PART 6 DISORDERS OF CARBOHYDRATE METABOLISM

57	Galactosemia	415
58	Glycogen storage diseases: introduction	425
59	Glycogenosis type I – Von Gierke disease	428
60	Glycogenosis type II/Pompe/lysosomal α -glucosidase deficiency	438
61	Glycogenosis type III/amylo-1,6-glucosidase (debrancher) deficiency	447

PART 7 PEROXISOMAL DISORDERS

62	Adrenoleukodystrophy	459
63	Neonatal adrenoleukodystrophy/disorders of peroxisomal biogenesis	469

PART 8 DISORDERS OF PURINE METABOLISM

64	Lesch-Nyhan disease and variants	483
65	Adenine phosphoribosyl-transferase deficiency	498
66	Phosphoribosylpyrophosphate synthetase and its abnormalities	503
67	Adenosine deaminase deficiency	507
68	Adenylosuccinate lyase deficiency	514
69	Orotic aciduria	518

PART 9 DISORDERS OF TRANSPORT AND MINERAL METABOLISM

70	Cystinuria	525
71	Cystinosis	532
72	Hartnup disease	540
73	Histidinuria	544
74	Menkes disease	546

PART 10 MUCOPOLYSACCHARIDOSES

75	Introduction to mucopolysaccharidoses	555
76	Hurler disease/mucopolysaccharidosis type IH α -L-iduronidase deficiency	558
77	Scheie and Hurler-Scheie diseases/mucopolysaccharidosis IS and IHS/ α -iduronidase deficiency	566
78	Hunter disease/mucopolysaccharidosis type II/iduronate sulfatase deficiency	572
79	Sanfilippo disease/mucopolysaccharidosis type III	580
80	Morquio syndrome/mucopolysaccharidosis type IV/keratan sulfatase deficiency	588
81	Maroteaux-Lamy disease/mucopolysaccharidosis VI/N-acetylgalactosamine-4-sulfatase deficiency	597
82	Sly disease/ β -glucuronidase deficiency/mucopolysaccharidosis VII	605

PART 11 MUCOLIPIDOSES

83	I-cell disease/mucopolipidosis II	613
84	Mucopolipidosis III/pseudo-Hurler polydystrophy/N-acetyl-glucosaminyl-l-phosphotransferase deficiency	621

PART 12 DISORDERS OF CHOLESTEROL AND NEUTRAL LIPID METABOLISM

85	Familial hypercholesterolemia	631
86	Mevalonic aciduria	642
87	Lipoprotein lipase deficiency/type I hyperlipoproteinemia	648

PART 13 LIPID STORAGE DISORDERS

88	Fabry disease	659
89	GM ₁ gangliosidosis/ β -galactosidase deficiency	666
90	Tay-Sachs disease/hexosaminidase A deficiency	678
91	Sandhoff disease/GM ₂ gangliosidosis/deficiency of hexosaminidase A and B/hex-B subunit deficiency	686
92	GM ₂ activator deficiency/GM ₂ gangliosidosis – deficiency of the activator protein	694
93	Gaucher disease	698
94	Niemann-Pick disease	708
95	Niemann-Pick type C disease/cholesterol-processing abnormality	718
96	Krabbe disease/galactosylceramide lipidosis/globoid cell leukodystrophy	726
97	Wolman disease/cholesteryl ester storage disease	733
98	Fucosidosis	740
99	α -Mannosidosis	745
100	Galactosialidosis	752
101	Metachromatic leukodystrophy	760
102	Multiple sulfatase deficiency	769

PART 14 MISCELLANEOUS

103	Congenital disorder of glycosylation, type Ia	781
104	Other forms of congenital disorders of glycosylation	787

105	α_1 -Antitrypsin deficiency	803
106	Canavan disease/aspartoacylase deficiency	811
107	Ethylmalonic encephalopathy	819
108	Disorders of creatine synthesis or transport	827
Appendix		833
Index		847

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Chapter 41

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Foreword

We must not cease from exploration,
And at the end of all our exploring,
Will be to arrive where we started,
And know the place for the first time.

T.S. Elliot, Four Quartets, 1942.

In 1942 only a handful of inborn errors of metabolism, sometimes called orphan diseases, were recognised with little to no treatment available. Destiny would take its course and genetic counselling was virtually all that could be offered. Phenylketonuria was then shown by Horst Bickel from the Children's Hospital, Heidelberg, Germany, to be a treatable "genetic" disease in which early diagnosis and dietary treatment prevented impaired mental development. Subsequently, many other inborn errors of metabolism became manageable in a similar way, i.e. with substrate deprivation strategies: maple syrup urine disease, galactosemia, fructosemia, tyrosinemia type 2, and others. Pharmacological doses of vitamins proved useful in defects of cobalamin and biotin metabolism, in distinct forms of homocystinuria, and some others. Avoiding of fasting was recognized as the cornerstone of successful therapy for defects of fatty acid oxidation, ketogenesis and glycogenolysis. Initially progress had been slow but has begun to explode over the last decennium as current progress in understanding the molecular and pathophysiological bases of inborn errors of metabolism funnels into the development of successful rational therapies: new treatment protocols - new therapeutic agents (drugs and foods) - improved tissue transplantation, enzyme replacement and gene therapy by other means.

The world health organisation (WHO) as well as the European Union (EU) have now announced genetic and orphan diseases as a major health challenge of the future. Among those the by now more than 500 inborn errors of metabolism are especially important because of their relatively high frequency and because successful rationale therapy is already available or will become so in the near future. As a group, they account for approx. 1 in 100 births worldwide. Scientific and technological advances offer enormous benefit to patients suffering from inborn errors of metabolism often completely preventing life-long burden and suffering. Early diagnosis by extended

newborn population screening with subsequent early treatment is the most successful approach. Tandem mass spectrometry has recently been implemented in newborn screening programs in an increasing number of countries and other diagnostic high-throughput techniques including primary molecular diagnostics are at the edge. Handicap and suffering can be prevented from thousands of children and their families. Although novel diagnostic and therapeutic possibilities never come for free, extended newborn screening is far more cost-effective than other medical advances. The costs of screening programs are greatly outnumbered by the costs for direct health and social costs in childhood.

The field of inborn errors of metabolism or Metabolic Medicine is continuing to increase, both by its size and fortunately even more by our knowledge. Clinical expertise and a good cooperation between the referring physician and the metabolic specialist and a broad spectrum of metabolic investigations in the respective center are the key to successful diagnosis and treatment. Each disease and each patient is different from each other. When molecular genetics came to medicine, there was a widely held belief, that knowing the genotype at the particular locus would predict the corresponding phenotype and assist counseling and treatment. It has become clear that this has been rather naive. Although genotype-phenotype correlation is strong in some diseases, there is a huge number of examples where the phenotype cannot be explained by the mutations found. Even more, it has become obvious that, in addition to mutations of the affected gene and environment, many other factors influence the phenotype. The role of numerous factors affecting post-transcriptional events, (including transport of RNA, protein synthesis, folding, and degradation.) and their mutual relationships are at best partly understood.

The "Atlas of Inherited Metabolic Diseases" is now set in its 3rd edition and has become the in-depth clinical reference resource for inborn errors of metabolism, combining in numerous details clinical presentation, treatment, monitoring and course. After brief but solid biochemical and molecular background information physicians will find the most comprehensive clinical reference book for Metabolic Medicine with instructive descriptions of clinical situations and the possibility of a visual double check on a metabolic syndrome with

physical characteristics through the great photos found nowhere else. The content of this book draws from decades long clinical experiences in its best way, always asking what could have been done better. It has been and will continue to be an invaluable source for metabolic physicians in

the care for their patients. Reflecting their experience in the details and advice found in the “Atlas of Inherited Metabolic Diseases” they may often find themselves remembering the beautiful lines of T.S. Elliot.

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Preface

This book is designed as a source of practical information of use in the diagnosis and management of patients with inherited diseases of metabolism. We have kept the focus, as did Garrod, on the inborn errors. This permits a unity of theme. At the same time, the reality is that genetically determined human variation in metabolism leads to an enormous variety of clinical expression crossing most of the boundaries of clinical subspecialty.

We want this book to be helpful to physicians at the bedside, in the intensive care unit, and in the clinics and offices, as well as to biochemical geneticists and clinical chemists involved in laboratory diagnosis. The atlas format has permitted us to include very many illustrations of patients. Metabolic pathways have been shown with a reductionist or high power view of just that area most relevant to each disease. In addition, the chapters deal with individual diseases. There are introductory chapters to the organic acidemias, the disorders of the urea cycle, the disorders of fatty acid oxidation, the lactic acidemias, the glycogenoses, and the mucopolysaccharidoses, which provide some general considerations of these areas of metabolism and permit us to avoid some redundancy. With these exceptions, each chapter represents defective activity of a single enzyme. Mutations in a single gene can lead to a very large family of different variant enzymes and accordingly very different clinical phenotypes. In general, we have considered this variation in each chapter, with emphasis on the most common expression. In two instances, we have given variants separate treatments. There is historical precedent for separate consideration of Hurler disease from the Scheie and Hurler-Scheie variants and for the separate consideration of mucopolipidoses II and III. We have continued that. In contrast, we now have an integrated chapter for HPRT deficiency.

The rates of discovery of new or previously unrecognized diseases in this field are enormous. In the 1980s, we saw for the first time descriptions of many of the currently known disorders of fatty acid oxidation; in the 1990s, we saw the numbers of known discrete mitochondrial DNA mutations increase rapidly. Some of these diseases are turning out to be relatively common. Medium-chain acyl CoA dehydrogenase (MCAD) deficiency occurs once in approximately 10,000 births, and most patients have the same mutation. On the other hand, although it is clear that in the aggregate the

inherited diseases of metabolism make up a sizeable portion of human morbidity and mortality, each individual disease tends to be rarely encountered. Even an expert may find years have elapsed since he last saw a patient with a given disorder, reviewed the literature, and ordered it in a way that would help with diagnosis or treatment. It helps to have the relevant information in one place for ready retrieval. This atlas serves that purpose for us. We are hopeful that it will do the same for our readers.

The advent of molecular biologic approaches to genetics and the increasing exploration of the human genome have changed forever the scope of human genetics and the manner in which it is practiced. In the atlas, we have endeavored to seek a balance among the molecular biology and the nature of mutation, the enzymology and intermediary metabolism, and clinical practice. Our focus is on the clinician. Algorithms are provided for the logical work up of a patient with lactic acidemia and disorders of fatty acid oxidation, and a systematic approach to the diagnosis of a patient with hyperammonemia.

Medical genetics is now officially recognized in many countries among clinical and laboratory specialties. Trainees preparing themselves for board examinations might want to read the atlas from cover to cover. We hope that in addition to medical geneticists, pediatricians, neurologists, internists, pathologists, and all those who interact with patients with these disorders will find the atlas of assistance in their practices.

The field is moving so rapidly it is an experience to keep current in any disease. There is much in this book that is new, different, or virtually unique. Certainly, the pictures are for us a resource. Mutations have now been identified in the genes for the very strange ethylmalonic aciduria whose petechial exacerbations lead regularly to treatment for meningococemia. The discovery of this gene, *ETHE1*, by homozygosity mapping, illustrates the powerful new influence of molecular biology and the data provided by the human genome project in this field.

In I-cell disease and pseudohurler polydystrophy, the basic defect is in the processing of lysosomal enzymes to permit their recognition and entry into cellular lysosomes. The fascinating and novel mechanism uncovered in the multiple sulfatase deficiency defect is in an enzyme which catalyzes a post-translational change of a cysteine moiety in each of the sulfatase enzymes to an amino-oxopropionic

acid moiety, which change normally converts inactive sulfatase proteins to catalytically active enzymes.

Among the challenges for diagnosis and management highlighted in this volume are the disorders of fatty acid oxidation and the lactic acidemias and mitochondrial disease. The latter includes the acronymic disorders resulting from mitochondrial DNA mutation and the Pearson syndrome, which may present in infancy as a pure hematologic disorder. It also includes the deficiency of DNA polymerase, which results in a mitochondrial DNA depletion syndrome. The disorders of creatine synthesis are a challenge for diagnosis. They are sometimes suspected when the urine is analyzed for organic acids and amino acids, and everything is high, because we base our analyses per mole of creatinine. They may be elegantly demonstrated by nuclear magnetic resonance spectroscopy (NMRS). It is turning out that these disorders in aggregate are as common as PKU and should be looked for in patients with nonspecific developmental delay.

The atlas was generated by our experience with patients

with metabolic disease. We are grateful to the many physicians who have referred these patients to us and to those who have shared their illustrations with us. We are appreciative of the help of many of our fellows and colleagues who have helped us care for and study these patients. They include Drs Nadia Sakati, Richard Hass, Fred Levine, Robert Naviaux, Jon Wolff, Mary Willis, Zarazuela Zolkipli, Ilya Gertsman, and Karen McGowan.

Original artwork was provided by Ms Michelle Williamson of BioMedical Design. Images of tandem mass spectrometry were recovered by Mr Jon Gangoiti of the Biochemical Genetics Laboratory at UCSD. We are particularly indebted to the work of many: Mrs Lilia Fernandez, Ms Sandra Hoffert, Ms Susan Allen, Dr Linh Vuong, and Mrs Elizabeth Taylor for the conversion of handwritten pages into polished typed electronic manuscript.

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ORGANIC ACIDEMIAS

1. Introduction	3
2. Propionic acidemia	8
3. Methylmalonic acidemia	19
4. Methylmalonic aciduria and homocystinuria (cobalamin C and D disease)	33
5. Multiple carboxylase deficiency/holocarboxylase synthetase deficiency	40
6. Multiple carboxylase deficiency/biotinidase deficiency	47
7. Isovaleric acidemia	57
8. Glutaric aciduria (type I)	64
9. 3-Methylcrotonyl CoA carboxylase deficiency/3-methylcrotonyl glycinuria	74
10. D-2-Hydroxyglutaric aciduria	79
11. L-2-Hydroxyglutaric aciduria	85
12. 4-Hydroxybutyric aciduria	89
13. Mitochondrial acetoacetyl-CoA thiolase (3-oxothiolase) deficiency	95

Introduction

The inborn errors of organic acid metabolism represent a spectrum of disorders, most of them relatively recently recognized. Many of them produce life-threatening illness very early in life. They should be suspected in any patient with metabolic acidosis, and certainly when there is an anion gap (Table 1.1). The variety of metabolic pathways involved is indicated in Figure 1.1.

The classic presentation of the organic acidemias is in infancy, often in the neonatal period, followed by recurrent episodes of metabolic decompensation, usually precipitated by infection. The infant begins vomiting and anorexic. This may be followed by the rapid deep breathing of acidosis. A ketotic odor may be appreciated. There may be rapid progression through lethargy to coma, or there may be convulsions. Hypothermia may be the only manifestation, besides failure to feed and lethargy. Further progression is to apnea and, in the absence of intubation and assisted ventilation, death.

Initial laboratory evaluation involves tests that are readily available in most clinical chemistry laboratories. Most important in early discrimination are the electrolytes and the ammonia. Blood gases are often the first data available in a very sick infant. Acidosis and hyperammonemia are indicative of an organic acidemia. In contrast, a patient with a urea cycle defect has hyperammonemia and alkalosis. It

is important not to delay treatment of acidosis in the belief that the problem is a urea cycle defect. Hyperammonemia regardless of cause must be treated. Hypocalcemia may be a nonspecific harbinger of metabolic disease. Elevated levels of lactate in the absence of cardiac disease, shock or hypoxemia are often seen in organic acidemias, as well as in the lactic acidemias of mitochondrial disease. The blood count is useful in indicating the presence or absence of infection. More important, neutropenia with or without thrombocytopenia or even with pancytopenia is characteristic of organic acidemia.

In the presence of acidosis suggesting organic aciduria, the assays of choice are organic acid analysis of the urine and acylcarnitine profile of the plasma.

A number of the organic acid disorders are on the catabolic pathways for the branched-chain amino acids, or other amino acids, but the site of the enzymatic defect is sufficiently removed from the step at which the amino group is lost that the amino acids do not accumulate, and thus these disorders are not detected by methods of amino acid analysis. They remained largely unrecognized until the development of methods of detection, particularly gas chromatography-mass spectrometry (GCMS) [1], that were of sufficient generality not to depend on a single functional group for detection. Quantitative organic analysis is an important aspect of this methodology. Tandem mass spectrometry (MS/MS) [2, 3] (Table 1.2) has added another important method of detection of organic acids as their carnitine esters; this methodology has made these diseases subjects for neonatal screening.

Gas chromatography-mass spectrometry has been the basis for monitoring levels of relevant metabolites in the course of management. Therapeutic intervention, including cofactor or other dosage and dietary restriction, are dependent on accurate knowledge of the concentrations of those compounds that accumulate behind the block. Tandem mass spectrometry may also serve this purpose. In general, therapeutic efficacy is best when concentrations of accumulated metabolite(s) are kept at the lowest achievable level. This is seldom zero, except in cofactor-responsive inborn errors, such as biotin-responsive multiple carboxylase deficiency (Chapters 5 and 6). More commonly, a plateau level of metabolite is achieved, at

Table 1.1 Mnemonic for the differential diagnosis of metabolic acidosis with an elevated anion gap (DIMPLES)

D	Diabetic ketoacidosis
I	Inborn error of metabolism, iron, isoniazid
M	Methanol, metformin
P	Paraldehyde, phenformin
L	Lactic acidemia
E	Ethanol, ethylene glycol
S	Salicylates, solvents, strychnine

The mnemonic has been written as mudpiles or mudpies, including u for uremia, but in clinical practice uremia tends to be recognized as early as the acidosis, making this unnecessary; the latter form leaves out lactic acidemia, an important omission. The current form highlights metabolic causes of acidosis.

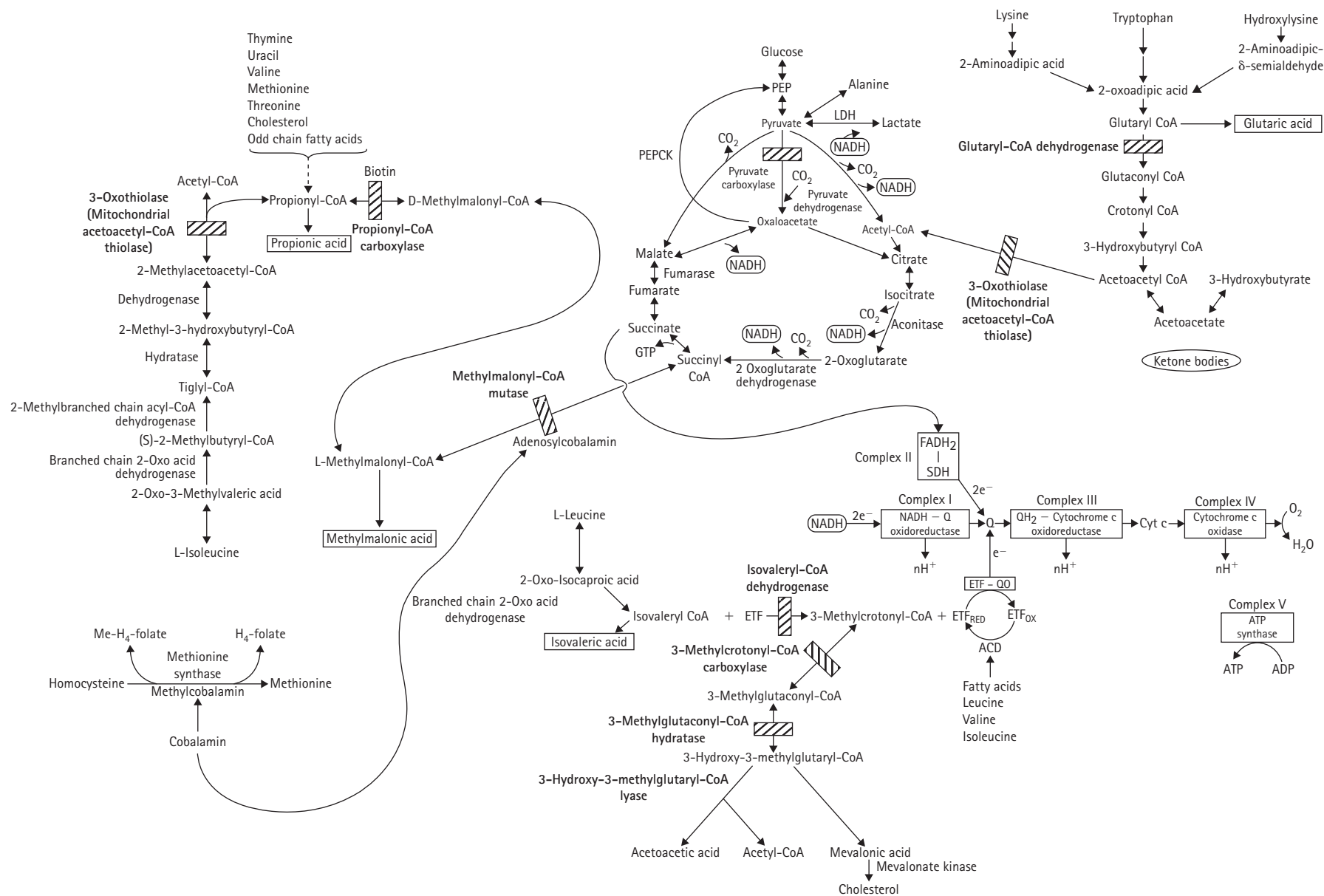


Figure 1.1 Metabolic interrelations of relevance to the organic acidemias. Many of these disorders are characterized by the accumulation of CoA esters. Many present with lactic acidemia.

Table 1.2 Acylcarnitine profiles of plasma in the diagnosis of organic acidemias

Disorder	Acylcarnitine	Control reference ^a	Patient
Propionic acidemia	C3	0.07–1.77	6.50–60.10
Methylmalonic acidemia	C3	0.07–1.77	13.00–90.50
	C4DC	0.00–0.04	0.12–0.94
Isobutyryl-CoA dehydrogenase deficiency	C4	0.06–1.05	
2-Oxothiolase deficiency	C5:1	0.00–0.10	0.14–0.72
	C5OH	0.01–0.11	0.12–0.30
Isovaleric acidemia	C5	0.06–0.62	52.96–60.47
Methylcrotonyl-CoA carboxylase deficiency (incl. maternal)	C5	0.06–0.62	15.52–18.38
	C5OH	0.01–0.11	0.80
Multiple carboxylase deficiency – holocarboxylase synthetase and biotinidase deficiencies	C5OH	0.06	
2-Methylbutyryl-CoA dehydrogenase deficiency	C5	0.06–0.52	1.4–2.4
Malonic aciduria	C3DC	0.05	
2-Methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency	C5OH	0.01–0.11	
	C5:1	0.00–0.51	
Glutaric acidemia	C5DC	3.00–0.10	0.46–1.34

Adapted from Vreken *et al.* [2] and Matern [3] and other sources.

^a95th percentile of the reference range.

DC, dicarboxylic acid.

which further restriction of metabolite intake leads to catabolism and an increase in metabolite accumulation, as well as impairment of weight gain and negative nitrogen balance. In disorders in which the organic acid is a product of amino acid metabolism, such as methylmalonic aciduria, we also measure concentrations of amino acids in plasma, and, while our patients have levels of the precursor amino acids much lower than those usually recommended as normal, we keep them above those at which weight gain stops or nitrogen balance becomes negative [3]. We maintain intake between such floor levels and a ceiling at which the plateau is exceeded and metabolite levels rise.

Quantification of organic acid analysis is essential for management; it may also be important in diagnosis. For instance, the presence of hydroxyisovalerate, hydroxypropionate, and methylcitrate may suggest a diagnosis of multiple carboxylase deficiency, but these compounds are also found in propionic acidemia. The two are readily distinguished by quantification. In multiple carboxylase deficiency, the amounts of hydroxyisovalerate are large and those of the other compounds small, while in propionic acidemia, the reverse is found. Misdiagnosis of propionic acidemia as multiple carboxylase deficiency has been catastrophic.

Other methodology has been applied to the detection of organic acids. Nuclear magnetic resonance (NMR) spectrometry has become available for these purposes, as the resolution of the machines has improved considerably [4]. The ability to test urine or other biological fluids without complex sample preparation raises the possibility of much more rapid diagnosis. Wider applicability should

reduce the cost of diagnostic procedures. The application of MS/MS [2, 5] to the detection of organic acidemias is of particular benefit in emergencies, for it shortens the time required for diagnosis.

Organic acid analysis and the occurrence of unique metabolites has led to highly accurate, rapid methods of prenatal diagnosis by GCMS of the amniotic fluid, especially with selected ion monitoring and stable isotope dilution internal standards [6]. Most experience is with analysis for methylcitrate and methylmalonic acids in the prenatal diagnosis of propionic acidemia and methylmalonic acidemia. Methodology is also available for the prenatal diagnosis of orotic aciduria [7], hepatorenal tyrosinemia [8], holocarboxylase synthetase deficiency [9], galactosemia [10], mevalonic acidemia [11], glutaryl CoA-dehydrogenase deficiency [12], and 4-hydroxybutyric aciduria [13].

Analysis of the organic acids of the urine may detect the presence of a disorder of neurotransmitter function, although the diagnosis is usually made by analysis of neurotransmitters or their products in cerebrospinal fluid (CSF) [14]. A patient with neonatal hypoglycemia and metabolic acidosis developed dystonia, oculogyric crises, and hypothermia at eight months. He was found on organic acid analysis of the urine to have increased levels of vanillic acid neonatally and later vanillic acid and acetylvannilalanine. Levels of these compounds in CSF were very high, while those of 5-hydroxyindolacetic acid and homovanillic acid were low. Enzyme assay revealed nearly undetectable aromatic L-amino acid decarboxylase activity [15].

Organic acid analysis is often confounded by the presence of compounds arising from intestinal bacterial metabolites, pharmacologic agents, nutritional supplements, or nutritional deficiency. A compendium of metabolites found on organic acid analysis in inborn errors of metabolism and in other situations has been published by Kumps *et al.* [16]. Some of the common confounding metabolites are shown in Table 1.3. Organic acid analysis is commonly ordered on patients during illness, and many illnesses are accompanied by ketosis with its elevated excretion of acetoacetate and 3-hydroxybutyrate. Accompanying ketosis are increases in the excretion of 3-hydroxyisovalerate, 3-hydroxyisobutyrate and dicarboxylic acids including long chain 3-hydroxy compounds. In this way, the pattern may be mistaken for long chain 3-hydroxyacyl CoA

dehydrogenase (LCHAD) deficiency (Chapter 40), but of course in LCHAD deficiency ketonuria is inappropriately low. This distinction rules out other disorders of fatty acid oxidation suggested by the dicarboxylic aciduria. In disorders of fatty acid oxidation ketonuria may be present, but the ratio of urinary adipic to 3-hydroxybutyric acid is >0.5 [17]. Lactic acidemia and lactic aciduria may also be confusing because of associated increase not only in pyruvic acid, but also the branched chain keto and hydroxy acids, as found in defects of the E3 subunit of the pyruvate dehydrogenase complex.

Bacterial metabolism in the intestine is another confounding variable, which becomes particularly prominent in malabsorptive syndromes. Among the compounds found in the urine is lactic acid; this is D-lactic acid, but

Table 1.3 Some organic acids found in the urine in the absence of inherited metabolic disease

Compound	Situation	Inborn error in which found
Adipic acid	Gelatin; fasting ketosis	Disorders of fatty acid oxidation
Furane derivatives: Dicarboxylate; Furoylglycine; 5-Hydroxymethyl-2-furoate	Heated sugars	
Glutaric acid	Intestinal bacterial metabolism	Glutaric aciduria I and II
Glycolic acid	Ethylene glycol poisoning	Hyperoxaluria type I; 4-Hydroxybutyric
3-Hydroxyadipic acid	Fasting	LCHAD deficiency
5-Hydroxyhexandic acid	MCT, Valproate, Ketosis	MCAD, MAD, VLCAD, LCHAD deficiency
5-Hydroxyhexanoic acid	MCT ingestion; ketosis	MCAD deficiency; multiple acyl CoA dehydrogenase deficiency
2-Hydroxyisocaproate	Short bowel syndrome (D-form)	Maple syrup urine disease (MSUD)
3-Hydroxyisovalerate	Ketosis; Valproic acid	Multiple carboxylase deficiency; isovaleric acidemia, lactic acidemia, 3-methylcrotonyl-CoA carboxylase deficiency
4-Hydroxyphenylacetate, lactate; or -pyruvate	Intestinal bacteria	Tyrosinemia; hawkinsinuria
2-Hydroxyphenylacetic	Uremia	Phenylketonuria; BH4 deficiency
2-Ketoglutaric acid	Urinary tract infection; infancy	2-Ketoglutaryl CoA dehydrogenase deficiency
3-Methylglutaconic acid	Uremia, pregnancy	Methylglutaconic aciduria, carbamyl phosphate synthetase deficiency
Methylmalonic acid	B12 deficiency; intestinal bacteria	Methylmalonic acidemia; transcobalamin II deficiency
N-acetyltyrosine	Parenteral solutions	Tyrosinemia
Oxalic acid	Intestinal malabsorption; idiopathic; pyridoxine deficiency; rhubarb, spinach, and other vegetables; ethylene glycol; ascorbic acid; methoxyflurane	Hyperoxalurias
5-Oxyproline (pyroglutamic acid)	Nonenzymatic conversion from glutamine in stored sample; vigabatrin; abnormal glycine metabolism; iron oxoprolinate	Pyroglutamic aciduria; hawkinsinuria; cystinosis
Phenylacetate; phenylacetylglutamine; phenyllactate; phenylpyruvate	Intestinal bacteria; treatment of urea cycle defects with phenylacetate or phenylbutyrate	Phenylketonuria
Vanillactic acid	Bananas; neuroblastoma; carbidopa	L-Amino acid decarboxylase deficiency

the chromatogram does not distinguish the D from the L forms. Specific enzymatic or other distinction must be made or the patient could be treated with oral neomycin or metronidazole and the urine reassayed. Other compounds resulting from intestinal bacteria are propionate metabolites, including methylmalonate, and aromatic compounds such as p-hydroxyphenylacetate, p-hydroxyphenyllactate, phenylacetylglutamine, phenylpropionylglycine, benzoate, and hippurate. Glutaric aciduria may also result from intestinal bacterial metabolism. Bacterial urinary tract infection also produces D-lactic aciduria; increased excretion of 2-oxoglutarate is characteristic; succinate and 3-hydroxypropionate may also be increased.

The administration of valproic acid yields a number of its metabolites, which may cause confusion, but their recognition permits understanding of the secondary effects the drug has on many areas of metabolism. Organic acids found in patients receiving the drug include 3-hydroxyisovalerate, 5-hydroxyhexanoate, p-hydroxyphenylpyruvate, hexanoylglycine, tiglylglycine, isovalerylglycine, and a variety of dicarboxylic acids.

Dicarboxylic aciduria is also a prominent result of the intake of medium chain triglyceride which is found increasingly in infant formulas. 5-Hydroxyhexanoate may serve as a clue, but other medium chain dicarboxylic acids, adipic, suberic, and sebacic are found. Large quantities of adipic acid are found in the urine of children eating gelatin.

REFERENCES

- Hoffmann G, Aramaki S, Blum-Hoffmann E *et al*. Quantitative analysis for organic acids in biological samples: batch isolation followed by gas chromatographic-mass spectrometric analysis. *Clin Chem* 1989; **38**: 587.
- Vreken P, van Lint AEM, Bootsma AH *et al*. Rapid diagnosis of organic acidemias and fatty acid oxidation defects by quantitative electrospray tandem-MS acylcarnitine analysis in plasma. In: Quant PA, Eaton S (eds). *Current Views of Fatty Acid Oxidation and Ketogenesis: From Organelles to Point Mutations*. New York: Plenum Publishers, 1999: 327–7.
- Matern D. Acylcarnitines, including *in vitro* loading tests. In: Blau N, Duran M, Gibson KM (eds). *Laboratory Guide to the Methods in Biochemical Genetics*. Berlin: Springer Verlag, 2008: 171–206.
- Nyhan WL. Disorders of propionate metabolism. In: Bickel H, Wachtel U (eds). *Inherited Diseases of Amino Acid Metabolism: Recent Progress in the Understanding, Recognition and Management*. New York: Thieme, 1985: 363–82.
- Lehnert W, Hunkler D. Possibilities of selective screening for inborn errors of metabolism using high-resolution 1H-FT-NMR spectrometry. *Eur J Pediatr* 1986; **145**: 260.
- Ozand PT, Rashed M, Gascon GG *et al*. Unusual presentations of propionic acidemia. *Brain Dev* 1994; **16**: 46.
- Sweetman L, Naylor G, Ladner T *et al*. Prenatal diagnosis of propionic and methylmalonic acidemia by stable isotope dilution analysis of methylcitric and methylmalonic acids in amniotic fluids. In: Schmidt H-L, Forstel H, Heinzinger K (eds). *Stable Isotopes*. Amsterdam: Elsevier Scientific, 1982: 287–93.
- Jakobs C, Sweetman L, Nyhan WL *et al*. Stable isotope dilution analysis of orotic acid and uracil in amniotic fluid. *Clin Chim Acta* 1984; **143**: 123.
- Jakobs C, Sweetman L, Nyhan WL. Chemical analysis of succinylacetone and 4-hydroxyphenylacetate in amniotic fluid using selective ion monitoring. *Prenat Diagn* 1984; **4**: 187.
- Jakobs C, Sweetman L, Nyhan WL, Packman S. Stable isotope dilution analysis of 3-hydroxyisovaleric acid in amniotic fluid: contribution to the prenatal diagnosis of inherited disorders of leucine catabolism. *J Inherit Metab Dis* 1984; **7**: 15.
- Jakobs C, Warner TB, Sweetman L, Nyhan WL. Stable isotope dilution analysis of galactitol in amniotic fluid: an accurate approach to the prenatal diagnosis of galactosemia. *Pediatr Res* 1984; **18**: 714.
- Gibson KM, Hoffmann G, Nyhan WL *et al*. Mevalonic aciduria: family studies in mevalonate kinase deficiency, an inborn error of cholesterol biosynthesis. *J Inherit Metab Dis* 1987; **10**: 282.
- Baric I, Wagner L, Feyh P *et al*. Sensitivity and specificity of free and total glutaric acid and 3-hydroxyglutaric acid measurements by stable-isotope dilution assays for the diagnosis of glutaric aciduria type I. *J Inherit Metab Dis* 1999; **22**: 867.
- Gibson KM, Aramaki S, Sweetman L *et al*. Stable isotope dilution analysis of 4-hydroxybutyric acid: an accurate method for quantification in physiological fluids and the prenatal diagnosis of 4-hydroxybutyric aciduria. *Biomed Environ Mass Spectrom* 1990; **19**: 89.
- Abdenur JE, Aheling NG, van Crucha AC *et al*. Aromatic L-amino acid decarboxylase (AADC) deficiency: unusual neonatal presentation and new findings in organic acid analysis (OA). *Am J Hum Genet* 2002; **71**: 424.
- Kumps A, Duez P, Mardens Y. Metabolic, nutritional, iatrogenic, and artifactual sources of urinary organic acids: a comprehensive table. *Clin Chem* 2002; **48**: 708.
- Treacy E, Pitt J, Egginton M, Hawkins R. Dicarboxylic aciduria, significance and prognostic indications. *Eur J Pediatr* 1994; **153**: 918.

Propionic acidemia

Introduction	8	Treatment	14
Clinical abnormalities	9	References	16
Genetics and pathogenesis	13		

MAJOR PHENOTYPIC EXPRESSION

Recurrent episodes of ketosis, acidosis and dehydration, progressive to coma; neutropenia, thrombocytopenia; osteoporosis; hyperglycinemia; propionic acidemia; methylcitraturia; and deficiency of propionyl CoA carboxylase.

INTRODUCTION

A patient with propionic acidemia was reported in 1961 [1] as having hyperglycinemia, a disorder of amino acid metabolism. Its most prominent feature was recurrent attacks of ketoacidosis. Analysis of the amino acids of blood and urine revealed very large quantities of glycine. Attacks were related to the intake of protein, and it was shown that ketonuria resulted regularly from the administration not of glycine, but of branched-chain amino acids and threonine and methionine [1, 2]. The discovery of a group of patients with hyperglycinemia who had none of these characteristics led us to coin the term 'nonketotic hyperglycinemia' (Chapter 22) to distinguish them from the original group that we called 'ketotic hyperglycinemia'. The discovery of methylmalonic acidemia in a group of patients who displayed the ketotic hyperglycinemia syndrome [3–5] led initially to the thought that all these patients had

methylmalonic acidemia. However, study of our initial patient and his sister, by Rosenberg and colleagues [6], indicated that neither excreted methylmalonic acid, and that they had propionic acidemia as a result of defective activity of propionyl CoA carboxylase (Figure 2.1). This enzyme is the first step in the pathway of propionate metabolism in which propionyl CoA, the product of the metabolism of isoleucine, valine, threonine, and methionine is converted to methylmalonyl CoA then to succinyl CoA and oxidation in the citric acid cycle.

The enzyme is composed of two subunits, α and β in an $\alpha_4\beta_4$ heteropolymeric complex. The apoenzyme is activated by the covalent binding of biotin to the amino group of lysine of the subunit. cDNA clones have been isolated for the α and β genes [7]. The α gene is on chromosome 13 and the β gene on chromosome 3. The nature of a number of mutations has been defined [8–10].

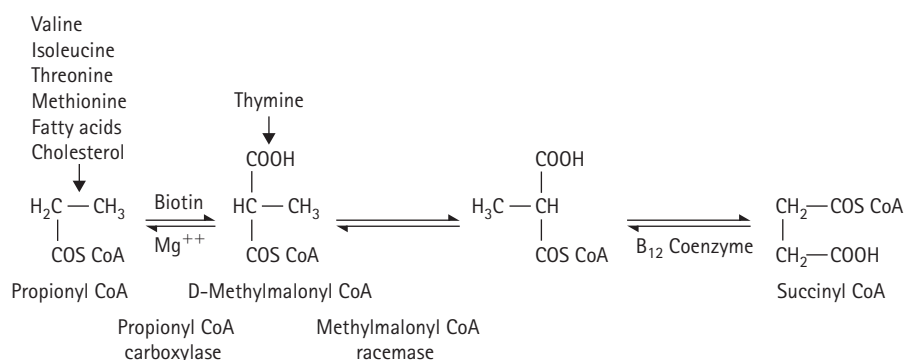


Figure 2.1 Metabolism of propionic acid. Propionyl CoA carboxylase is the site of the defect in propionic acidemia.



Figure 2.2 C: An infant with overwhelming illness.

CLINICAL ABNORMALITIES

Patients with propionic acidemia usually present first with life-threatening illness very early in life ([Figure 2.2](#)). Many patients have died in the course of one of these episodes of illness. Patients with metabolic disease, which presents this way in the neonatal period, may appear to have sepsis, ventricular hemorrhage or some other catastrophic process. It is likely that most patients die undiagnosed. A typical episode is heralded by ketonuria. The initial symptom is often vomiting, and some patients have had such impressive vomiting that they have been operated on with a diagnosis of pyloric stenosis [1, 11, 12]. Massive ketosis leads to acidosis and dehydration. Lethargy is progressive to coma. Unless the patient is treated vigorously with intubation and assisted ventilation, as well as very large quantities of fluid and electrolytes, shock intervenes and the outcome is death [13]. Presentation of a gravely ill infant can be with hypothermia. In an experience with 30 patients [14], 90 percent presented with severe acidosis.

Ketotic episodes are recurrent. They often follow infection, and, furthermore, at least in infancy, the untreated patient appears to be unusually susceptible to infection. We have seen a number of patients in whom septicemia, especially with *klebsiella*, has been documented ([Figure 2.2](#)). Initial presentations in some patients may mimic an immunodeficiency disease. Episodes are also related to diet; patients are intolerant of the usual dietary quantities of protein. A recurrent pattern of illness follows admission to hospital, correction of acidosis, and a period of no protein intake, after which the patient appears well. Feeding of the usual quantity of protein is reinitiated and

the patient sent home, where ketosis recurs as soon as toxic quantities of intermediates have reaccumulated.

Clinical chemistry reveals dramatic acidosis during the acute episodes. Arterial pH values as low as 6.9 may be seen, and the serum bicarbonate may be as low as 5 mEq/L or less. There is an anion gap. To some extent, this reflects the propionic acidemia, and there is lactic acid accumulation as well, but most of the acidosis results from accumulation of 3-hydroxybutyrate and acetoacetate. Symptomatic hypoglycemia may occur.

Some neonatal presentations of propionic acidemia are with hyperammonemia and coma, suggesting a disorder of the urea cycle; ammonia levels well over 1000 μM are not unusual. Most patients have typical ketoacidosis at this time, but some do not, making the differential diagnosis difficult. The presence of neutropenia and thrombocytopenia may provide a clue to the presence of an organic acidemia, and some infants have pancytopenia ([Figure 2.3](#)). Amino acid analysis reveals the typical elevation of glycine, as well as of glutamine in the hyperammonemic patient. Interestingly, episodes of recurrent illness after infancy almost never lead to clinically significant elevation of ammonia.

Infants with propionic acidemia are impressively hypotonic, and this may lead to delay in achieving developmental milestones even in patients that are ultimately developmentally normal. Our initial patient had impaired mental development and microcephaly [15]. Many of these patients have impaired mental development [16, 17]. Despite mild to moderate cognitive impairment, focal neurologic abnormalities appear to be rare [17]. Atrophy



Figure 2.3 LS: A four-year-old girl with propionic acidemia. Despite a neonatal presentation, at an evaluation at 18 years of age she was normal cognitively. However, she died in a typical ketoacidotic episode at 31 years.

has been observed on magnetic resonance imaging (MRI) of the brain [17]. Seizures and abnormalities of the electroencephalogram (EEG) have been observed. Of 11 early onset patients reported by Surtees *et al.* [18], all died; ages at death ranged from 6 days to eight years. No patient had an IQ greater than 60. Among nine patients with later onset (6 weeks to 24 months) two died, and all had IQs greater than 60.

We have thought that the cognitive and neurologic sequelae in this disease were more likely consequences of repeated overwhelming illness early in life, with attendant shock and diminished perfusion of the brain, than of the metabolic abnormality directly. This was consistent with experience with patients treated promptly and effectively who went on to develop normally into their teens (Figure 2.3) and with a few adult patients (Figure 2.4). The sister of the first patient was diagnosed prior to the development of any symptoms, and protein restriction was initiated immediately and carried out effectively [19]. Despite the occurrence of ketoacidosis with infection she developed normally and was intellectually fine at most recent report at over 30 years of age. Some of the patients of Surtees *et al.* [18] were of normal intelligence. One was diagnosed presymptomatically because his brother, whose onset was at 13 months, had the disease, and the presymptomatically



Figure 2.4 KZ: A 22-year-old Costa Rican girl with propionic acidemia. Two previous siblings had died with identical symptoms to those that she presented with in the early months of life. One sibling was operated on for pyloric stenosis, but at surgery, the pylorus was deemed normal. The patient's presentation included multiple episodes of metabolic ketoacidosis requiring admission to hospital following diagnosis at two years. There were no further admissions for acidotic imbalance. Since this picture was taken we have been informed that she died.

diagnosed brother was alive and of normal intelligence and neurologic examination at one year of age. Hyperammonemia over 200 μM was found in four of the early onset group and only one of the late onset group (the brother of the presymptomatically diagnosed patient).

Nevertheless, a small population of patients with propionic acidemia has had a virtually exclusively neurologic presentation, sometimes without much ketoacidosis. Hypertonia may follow hypotonia or hypotonia may persist (Figure 2.5). Choreoathetosis and dystonic posturing have been observed. Deep tendon reflexes are exaggerated and the Babinski response may be present.

In two patients with an exclusively neurologic presentation [20], the life-threatening episodes of ketoacidosis that usually serve as alerting signals were absent. In addition, hyperammonemia was prominent in late infancy in one and as late as 15 years in the other. Hypotonia, spastic quadriparesis and choreoathetosis were major manifestations. One patient displayed self-injurious behavior with mutilation of his lower lip (Figure 2.6). Choreoathetosis, pyramidal tract signs and dystonia have also been reported in other patients [19], including an infant who did not have ketoacidosis or hyperammonemia [21].

An infant who presented with a pure hyperammonemia picture without ketoacidosis is shown in Figure 2.7. MRI of the brain revealed extensive atrophy (Figure 2.8). An unusual patient [22] was diagnosed at 31 years of age after admission to a psychiatric hospital where he was admitted for bizarre behavior and studied further because of involuntary movements. We have observed MRI evidence of hypodense myelin, along with areas of increased signal in the basal ganglia [20]. We have also encountered



Figure 2.5 A four-year-old Saudi patient with propionic acidemia who was still impressively hypotonic.



Figure 2.6 A 20-year-old man with propionic acidemia who presented with severe impairment of cognitive function, spastic quadriplegia and a mutilated lip that led to his referral as a patient with Lesch-Nyhan disease. HPRT assay was normal and metabolic exploration led to the diagnosis.



Figure 2.7 An infant with propionic acidemia who presented acutely at 20 days of age in coma with a blood ammonia of 450 μmol/L and no ketoacidosis. A brother had died at 40 days after an identical clinical presentation.

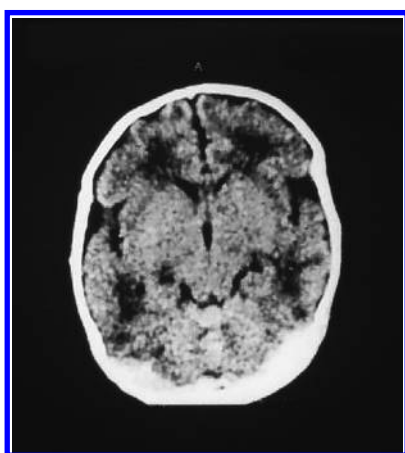


Figure 2.8 Magnetic resonance image of the brain of the infant in Figure 2.7, illustrating extensive cerebral atrophy. (Illustration and Figure 2.8 were kindly provided by Dr I Baric of the University Hospital Center, Zagreb, Croatia.)

a metabolic stroke in an eight-year-old patient with propionic acidemia in which there was virtually complete infarction of the basal ganglia followed by death [23, 24]. We have been informed about a similar patient who did not die, but remained in a vegetative state. A 15-year-old diagnosed neonatally suddenly developed a stroke of the basal ganglia from which he ultimately recovered [25]. Assessment of cerebral vessels showed no abnormality. Treatment with L-DOPA appeared to be beneficial.

Patients with propionic acidemia also regularly have neutropenia at the time of diagnosis. It is responsive to treatment of propionic acidemia (*vide infra*) and may reappear with recurrent metabolic imbalance. Transient thrombocytopenia is seen in infancy. Rarely, there may be anemia [26]. These hematological effects mirror the effects of propionyl CoA on marrow cell development, and they respond to metabolic control. Chronic moniliasis occurs in this syndrome, as well as in methylmalonic acidemia. This problem reflects the effect of propionyl CoA on T cell number and function and particularly their response to candida [27, 28]. In the series of Lehnert *et al.* [14] skin lesions were found in 53 percent; in addition to candida they encountered staphylococcal scalded skin syndrome, alopecia in two patients, and flaky or lamellar desquamation around the mouth or perineum that was called 'dermatitis acidemica'. In our experience, most of these noncandidal skin problems could be attributed to deficiency of protein or a specific amino acid often in a patient under excellent control who suddenly developed infection.

Osteoporosis is a regular concomitant of this disease and may be so severe that pathological fractures occur [2]. Diminished bone density may be documented even in patients maintained in excellent metabolic control.

Acute and recurrent pancreatitis has been observed as a complication of this disease [23], as well as other organic acidemias. In these patients, vomiting and abdominal pains are associated with elevated levels of amylase and lipase.

For reasons that are not clear, patients have been observed who have no symptoms of disease, at least to the time of the report at teenage, despite documentation of virtually no enzyme activity and ascertainment through symptomatic siblings [29]. We have not encountered such patients, nor have those reporting experience with large numbers of patients [14, 30].

Infants with propionic acidemia tend to resemble each other and those with methylmalonic acidemia (Figure 2.9). Characteristic facial features are: frontal bossing; widened depressed nasal bridge, and an appearance of wide-set eyes; epicanthal folds, and a long filtrum with upward curvature of the lips. In addition, the nipples may be hypoplastic or inverted (Figure 2.10).

Neuropathologic findings [31, 32] in patients dying in the neonatal period have been those of spongy degeneration of the white matter. In patients dying later, abnormalities in the basal ganglia were prominent [23, 31]. These included gross shrinkage and marbling, as well as microscopic neuronal loss and gliosis.

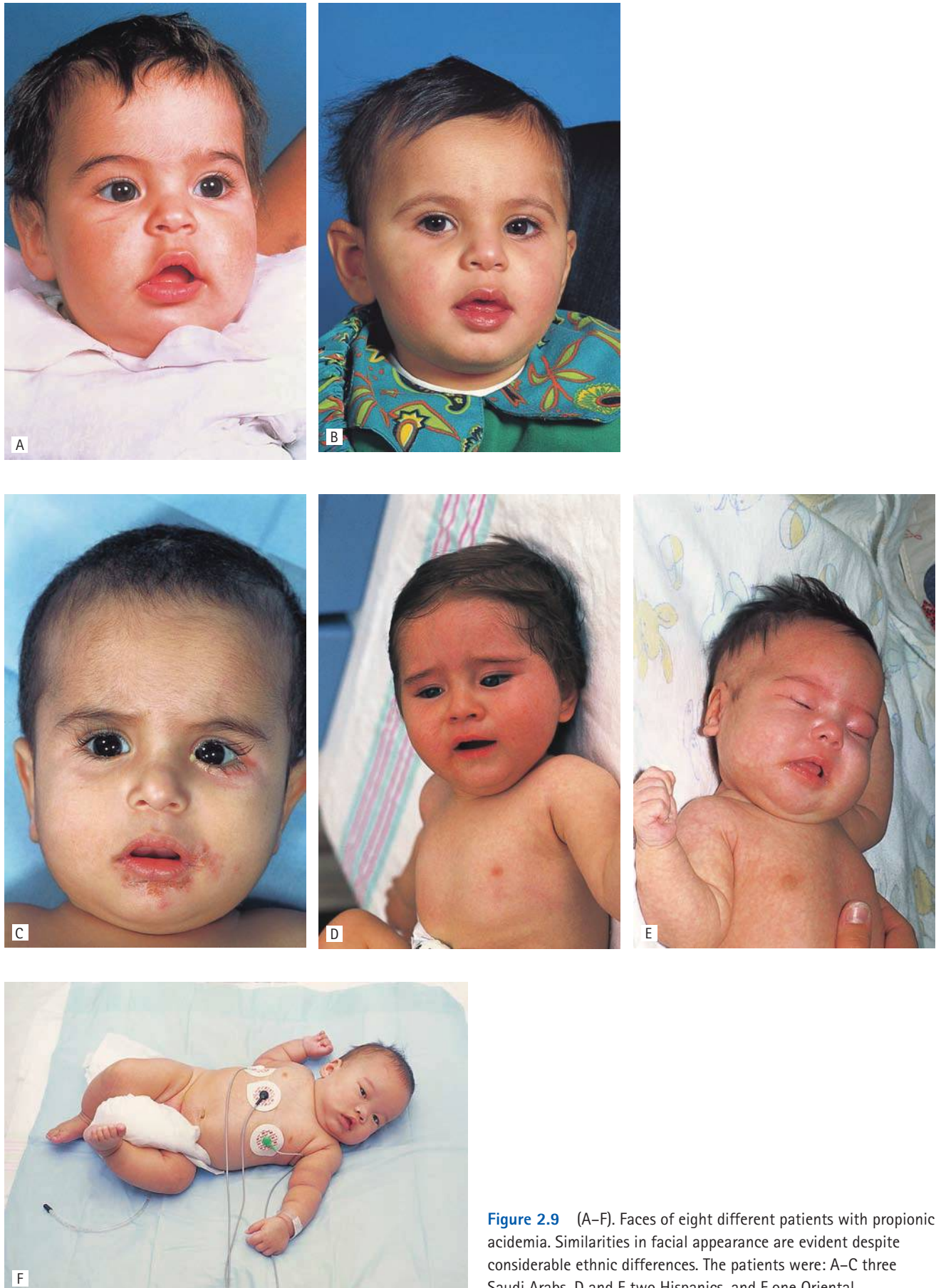


Figure 2.9 (A–F). Faces of eight different patients with propionic acidemia. Similarities in facial appearance are evident despite considerable ethnic differences. The patients were: A–C three Saudi Arabs, D and E two Hispanics, and F one Oriental.



Figure 2.10 Inverted nipples in a patient with propionic acidemia.

Among late complications of inherited metabolic diseases, cardiomyopathy is emerging as a major complication of propionic acidemia [32–35]. Clearly, this may be fatal. Fatal hypertrophic cardiomyopathy was found at autopsy in a patient despite therapy with carnitine and absence of an acute episode of decompensation [34]. Concentrations of carnitine in cardiac muscle were found to be low.

GENETICS AND PATHOGENESIS

Propionic acidemia is inherited as an autosomal recessive trait. The enzymatic site of the defect is propionyl CoA carboxylase [36, 37]. Activity in extracts of leukocytes and fibroblasts is very low, usually less than 5 percent of control (Table 2.1). Studies with somatic cell hybrids have provided evidence of two complementation groups, PccA and PccBC, which correspond to abnormalities in the α and β subunits, respectively [38–43]. The BC group contains two subgroups, B and C, in which intragroup complementation

is thought to be interallelic. Patients in the A subgroup have mutations in the A gene for the α chain, and those in the BC groups have mutations in the B gene for the β chain. Residual activity of propionyl CoA carboxylase correlates poorly with severity of disease or outcome [14].

Heterozygosity is not reliably determined by assay of the enzyme in cultured fibroblasts. A positive indicates heterozygosity, but a negative may not be consistent with its absence. Heterozygotes for the PccA group display approximately 50 percent of control activity of the enzyme, but those of the PccBC group are not distinguishable from normal [38].

Immunochemical assay of the PccA group has revealed many with little or no α chain of the enzyme [39] and other studies indicated an absence of α chain mRNA; these cells lack the subunit which is thought to have been degraded while the β chain mRNA was present [43]. This is consistent with the expression of 50 percent of activity in heterozygotes. Cells of the BC groups may contain immunoprecipitable subunits but lack β subunits [44, 45]. The normal activity in BC heterozygotes is thought to result from a five-fold greater synthesis of β subunits than α units. The amount of residual carboxylase activity measured in patients is thought to reflect the activity of other carboxylases on the substrate.

The cDNAs for the α [46] and β [47, 48] subunits have been cloned, and the genes have been mapped, respectively, to chromosomes 13q32 [49] and 3q13.3-22 [50]. The tetrapeptide sequence, Ala-Met-Lys-Met in the amino acid sequence of the chain deduced from the gene [7] appears to be a universal feature of the binding site of all carboxylases.

A number of mutations has been defined at the level of the DNA [9, 10, 48, 51–53]. Among mutations in the A gene, nonsense and splicing mutations, which cause exon skipping and deletions, have led commonly to an absence of mRNA [54]. Among point mutations in this gene, abolition of biotin binding was common [55, 56]. Among mutations in the B gene, there has been a number of missense mutations, such as C to T change, that changed an arginine at residue 410 of the β subunit to a tryptophan [52], which was common in Japanese patients; and an insertion/deletion (1218del14ins12) with a frame shift and a stop codon, that has been common in Caucasian cell lines studied [9, 51]. A frequent mutation in Spanish patients was 1170insT [57]. However, the 1218del14ins12 was found in 31 percent of Spanish and 44 percent of Latin American alleles [8, 57].

Prenatal diagnosis [58–62] has been accomplished by measurement of activity of propionyl CoA carboxylase in cultured amniotic fluid cells [58] or chorionic villus cells [59], or fixation of ^{14}C propionate in amniocytes [60]. It is more rapidly accomplished by the direct gas chromatography-mass spectrometry (GCMS) assay of methylcitric acid in amniotic fluid [61], a method which obviates the error always implicit in cell culture approaches that those cells ultimately analyzed are maternal, not fetal [62]. It has also been accomplished by measurement of

Table 2.1 Propionyl-CoA carboxylase activity (picomol of ^{14}C bicarbonate fixed/mg protein/min) in patients with propionic acidemia

	Normal	Patients
Lymphocytes		
Mean \pm 1 SD	232 \pm 87	10 \pm 9
(Range)	(160–447)	(0–36)
n	45	23
Fibroblasts		
Mean \pm 1 SE	294 \pm 94	15 \pm 17
(Range)	(128–537)	(0–51)
n	36	10

propionylcarnitine in amniotic fluid. In those families in which the mutation is known, it may be made by assay of the DNA, ideally with oligonucleotide probes.

There are a number of biochemical consequences of the defective activity of propionyl CoA carboxylase, many of which have direct relevance to the pathogenesis of the clinical manifestations of the disease. The immediately apparent consequence (Figure 2.1) is the inability to catabolize four essential amino acids: isoleucine, valine, threonine, and methionine. These amino acids are responsible for the toxicity of protein ingested in amounts greater than required for growth, and they were shown in the initial studies [1, 2] to induce ketonuria when administered individually.

Patients with propionic acidemia have elevated concentrations of glycine in the blood and urine. This was the first of the biochemical abnormalities to be recognized [1]. It occurs along with abnormal ketogenesis, also in methylmalonic acidemia (Chapter 3), in isovaleric acidemia (Chapter 7), and in 3-oxothiolase deficiency (Chapter 13).

The mechanism of hyperglycinemia appears to be an inhibition by propionyl CoA of the synthesis of the glycine cleaving enzyme leading to defective oxidation of glycine [63]. The hyperglycinemia of propionic acidemia is usually readily differentiated from nonketotic hyperglycinemia by the occurrence of episodes of ketosis. However, we have observed overwhelming illness without ketosis in a patient with propionic acidemia [64]. It is for this reason that all hyperglycinemic infants should be assessed for a possible diagnosis of propionic acidemia before a diagnosis of nonketotic hyperglycinemia is made.

When propionyl CoA accumulates, other metabolic products are found in the blood and urine. The predominant compound is 3-hydroxypropionic acid; others include tiglic acid, tiglylglycine, butanone, and propionylglycine. In addition, the unusual metabolite methylcitrate is formed by condensation of propionyl CoA and oxaloacetic acid [65]. This compound is an end product of metabolism and is very stable, resistant to conditions of shipment and bacterial contamination. In our hands, it is the most reliable chemical indicator of the presence of this disorder. It is useful in prenatal diagnosis, as well as the initial diagnosis. Odd chain fatty acids may accumulate in body lipids as a consequence of synthesis from propionyl CoA. They may be demonstrated and quantified in erythrocytes [66]. 3-Ureidopropionate is found in the urine [67], a consequence of propionate inhibition of ureidopropionase. The manifestations of patients with inherited deficiency of this enzyme of pyrimidine metabolism are reminiscent of those of propionic acidemic patients with changes in the basal ganglia, and there is *in vitro* evidence that ureidopropionate is neurotoxic [68]. 2-Methyl-3-oxovaleric acid, a product of self-condensation of two molecules of propionyl CoA, has been a useful metabolite for Lehnert and colleagues [14] for the diagnosis of propionic acidemia. Its reduction yields 3-hydroxy 2-methylvaleric acid. Hyperlysinemia or

hyperlysinuria encountered in propionic acidemia [14] appears to reflect study during hyperammonemia, during which lysine accumulates.

Abnormal ketogenesis is a major cause of morbidity and mortality in this disease. It could result from a variety of mechanisms. Propionic acid is an inhibitor of mitochondrial oxidation of succinic and 2-ketoglutaric acid, and propionyl CoA is an inhibitor of succinate: CoA ligase, and malate dehydrogenase [69]. Carnitine prevents this, consistent with its role in therapy. Carnitine is depleted in these patients, because it forms the propionylcarnitine ester, which is excreted in the urine. Analysis for propionylcarnitine has also been used for diagnosis, and has been effectively explored in prenatal diagnosis [70]. The accumulation of propionyl CoA, and its condensation with oxalacetate to form methylcitrate depletes oxalacetate and so acetyl CoA, deprived of substrate with which to condense to form citrate, condenses with itself to form acetoacetate. A variety of mitochondrial oxidative functions have been found to be inhibited by propionic CoA [71] here including pyruvate dehydrogenase, 2-ketoglutarate dehydrogenase and decrease in the amount and activities of the OXPHOS complexes I–IV.

The hyperammonemia observed in infants with propionic acidemia is a consequence of the inhibition of the urea cycle at the carbamylphosphate synthetase (CPS) step by propionyl CoA. This results from a competitive inhibition of N-acetylglutamate synthetase [72].

The obligatory biotin cofactor has led to the possibility that some patients with propionic acidemia are biotin responsive [73]. Nevertheless, no patient has been shown to be clinically responsive to biotin. Most patients we have tested by assessing the conversion of ^{13}C -propionate to $^{13}\text{CO}_2$ *in vivo*, before and after biotin, have shown no evidence of response [74]. The one patient in whom there was a small response had no clinical response to a course of treatment with biotin.

TREATMENT

The advent of programs of expanded newborn screening using tandem mass spectrometry has greatly increased the yield of patients with propionic acids treated presymptomatically. It has seemed likely that mutations that convey an attenuated clinical disease may be uncovered in this way, as has been observed in other disorders, such as medium chain acyl CoA dehydrogenase deficiency (Chapter 38), and 3-methylcrotonyl CoA carboxylase deficiency (Chapter 5), but the incidence of propionic acidemia in newborn screening does not appear to differ from incidence encountered in patients diagnosed clinically. The diagnostic analyte in dried neonatal blood spots is C3 (propionyl) carnitine. The ratio of C3/C0 is also elevated.

The cornerstone of treatment is the dietary restriction of the intake of all of those amino acids whose metabolism

takes them through propionyl CoA to the amounts that are required for growth and no more. We have provided these amino acids in the form of a standard cow's milk formula whose amino acid content is known. We have made up the rest of the calories in fat and carbohydrate [75–77]. It is not necessary to supply a mixture of the other amino acids, although this approach has regularly been employed; it is certainly indicated if an individual amino acid becomes limiting, but it is possible to manage such a patient by supplementing just that amino acid. Treatment must be monitored from time to time with quantitative assays of the relevant metabolites in the urine.

We also assess growth in weight, nitrogen balance, and the concentrations of amino acids in blood. We aim for an intake of protein below that at which plateau levels of amino acids rise and above the levels required for positive nitrogen balance, and growth and height [77]. The quantification of urinary urea [78] is a useful adjunct to the therapy. It may be useful to monitor erythrocyte concentrations of odd chain fatty acids [79]. We teach our parents to test for ketones in the urine using ketostix. Ideally, the urine is tested daily in infancy. Thereafter, it can be done at intervals, with special attention to periods of intercurrent infection.

The addition of carnitine to the therapeutic management of infants with this disease has had a major impact on management [80–84]. Patients are all carnitine-depleted in the absence of treatment. Treatment increases the excretion of carnitine esters, which should promote detoxification. It also substantially reduces the propensity for ketogenesis as tested by fasting [84]. Concomitantly, it has seemed that our patients tolerate the catabolism of infection better, and require less frequent admission to hospital. Doses generally employed have been from 60 to 100 mg/kg, although ketogenesis is less with 200 mg/kg. Doses higher than this usually produce diarrhea, but, otherwise, toxicity has not been encountered. Much higher doses can be employed parenterally without producing diarrhea.

Experience has indicated that the anabolic properties of human growth hormone have decreased the propensity for catabolism in these patients [85]. Certainly there is improvement in growth, lean body mass, and mineralization of bone, as well as decrease in adiposity.

The treatment of the acute episode of ketoacidosis requires vigorous attention to supportive therapy. We use very large amounts of parenteral fluid and electrolytes, along with high doses of intravenous carnitine (Table 2.2). Emergency is such that blood is obtained to determine concentrations at electrolytes and bicarbonate and an

intravenous infusion is started before taking time to take a history and do a physical examination. Following a bolus of 20 mL/kg of ringer lactate or isotonic saline we usually begin with isotonic (150 mEq/L) of NaHCO_3 . Carnitine is infused at a rate of 300 mg/kg. Electrolytes are determined at least every 6 hours. The serum concentration of sodium should be greater or equal 138 mmol/L. The NaHCO_3 content of the fluid may be reduced (to 75 MEq/L) after the serum bicarbonate has become normal, but it is continued at the same rate until the ketonuria has receded to small.

Fasting has been demonstrated [86] to increase the excretion of urinary metabolites of propionate, presumably from the oxidation of odd chain fatty acids stored in lipid. Consistent with this, studies of sources of propionate in patients with propionic acid and methylmalonic acidemia by means of ^{13}C -propionate turnover [87] indicated about 30 percent of propionate production not accounted for, suggesting that this much might come from propionate stored in lipid. Data are not available for propionate turnover in infants and children not subjected to overnight fast, which these authors had shown to increase excretion of urinary metabolites of propionate. The avoidance of fasting is recommended in this disorder. This is also an argument for the inclusion of glucose in the infusion solution; larger amounts may be beneficial. In a conscious patient without intestinal intolerance, cornstarch or polycose by mouth or nasogastric tube may be useful.

Neonatal hyperammonemia may require treatment with intravenous sodium benzoate and or phenylacetate (Chapter 23), or hemodialysis [88]. Carbamylglutamate, an activator of urea cycle activity of the N-acetylglutamate synthesis/carbamylphosphate synthetase area, has been shown to reduce hyperammonemia in this disease [89, 90], as well as in methylmalonic acidemia.

Parenteral mixtures of amino acids, in which the concentration of isoleucine, valine, threonine, and methionine are reduced or absent, may be useful, especially in the patient with intestinal abnormalities [91]. Insulin may be a useful adjunct [92]. The efficacy of growth hormone in the acute episode has not been assessed.

Studies of propionate production before and after treatment with metronidazole in three patients with propionic acidemia and three with methylmalonic acidemia [93] indicated that a mean of 22 percent could be attributed to formation of propionate by intestinal bacteria. However, the data were quite variable. In the patient with methylmalonic acidemia with the lowest level of methylmalonate excretion in the urine, the excretion after metronidazole was little changed, and the propionate turnover changed only from 46 to 41 $\mu\text{mol/kg}$ per hour, which does not seem significant. Similarly, these authors reported an average reduction of excretion of propionate metabolites of 41 percent in nine patients with disorders of propionate metabolism treated with metronidazole [86].

In our experience, results have been quite variable as measured by change in metabolite excretion following treatment with oral neomycin or metronidazole even

Table 2.2 Management of the acute episode of ketoacidosis (intravenous)

Water (mL/kg)	NaHCO_2 (mEq/L)	Glucose (%)	Carnitine (mg/kg)
200	150	5–10	300

in the same patient, suggesting that the intestine may be colonized by varying clones or groups of organisms, which do or do not make propionate. The reported data [86] are consistent with this, in that two patients with low levels of metabolites excreted had barely appreciable changes after metronidazole from 1.9 to 1.6 $\mu\text{mol/kg}$ per hour total metabolites and 4.0 to 2.9 even though the percentage decreases were 16 and 29 percent. A trial of antibiotic treatment is always worthwhile, and it is especially indicated by a change in excretion in a patient whose pattern is well known, and there is no obvious cause for catabolism. We prefer to start with neomycin because it is not absorbed. We have used a dose of 50 mg/kg. Metronidazole has been used in doses of 10–20 mg/kg.

Transplantation of the liver has been employed in propionic acidemia [94, 95]. As overall results of the procedure in children have improved, the results in propionic acidemia have become more encouraging. The metabolic abnormality is not corrected, but there may be a five-fold decrease in methylcitrate excretion, and there may be a major reduction in propensity to ketoacidosis.

REFERENCES

- Childs B, Nyhan WL, Borden MA *et al.* Idiopathic hyperglycinemia and hyperglycinuria, a new disorder of amino acid metabolism. *Pediatrics* 1961; **27**: 522.
- Childs B, Nyhan WL. Further observations of a patient with hyperglycinemia. *Pediatrics* 1964; **33**: 403.
- Oberholzer VC, Levin B, Burgess EA, Young WF. Methylmalonic aciduria: an inborn error of metabolism leading to chronic metabolic acidosis. *Arch Dis Child* 1967; **42**: 492.
- Stokke O, Eldjarn L, Norum KR *et al.* Methylmalonic aciduria: a new inborn error of metabolism which may cause fatal acidosis in the neonatal period. *Scand J Clin Lab Invest* 1967; **20**: 313.
- Rosenberg LE, Lilljeqvist A-C, Hsia YE. Methylmalonic aciduria: An inborn error leading to metabolic acidosis, long-chain ketonuria and intermittent hyperemia. *N Eng J Med* 1968; **278**: 1319.
- Hsia YE, Scully KJ, Rosenberg LE. Defective propionate carboxylation in ketotic hyperglycinaemia. *Lancet* 1969; **1**: 757.
- Lamhonwah A-M, Barankiewicz TJ, Willard HF *et al.* Isolation of cDNA clones coding for the α and β chains of human propionyl-CoA carboxylase: chromosomal assignments and DNA polymorphisms associated with PCCA and PCCB genes. *Proc Natl Acad Sci USA* 1986; **83**: 4864.
- Rodriguez-Pombo P, Hoenicka J, Muro S *et al.* Human propionyl-CoA carboxylase beta subunit gene. Exon-intron definition and mutation spectrum in Spanish and Latin American propionic acidemia. *Am J Hum Genet* 1998; **63**: 360.
- Lamhonwah AM, Troxel CE, Schuster S, Gravel RA. Two distinct mutations at the same site in the PCCB gene in propionic acidemia. *Genomics* 1990; **8**: 249.
- Desviat LR, Perez B, Perez-Cerda C *et al.* Propionic acidemia: mutation update and functional and structural effects of the variant alleles. *Mol Gen Metab* 2004; **83**: 28–37.
- Nyhan WL. Patterns of clinical expression and genetic variation in inborn errors of metabolism. In: Nyhan WL (ed.). *Heritable Disorders of Amino Acid Metabolism*. New York: John Wiley & Sons, 1974: 3–14.
- Nyhan WL. Introduction. In: Nyhan WL (ed.). *Abnormalities in Amino Acid Metabolism in Clinical Medicine*. Norwalk, CT: Appleton Century Crofts, 1984: 3–18.
- Hommes FA, Kuipers JRG, Elema JD *et al.* Propionic acidemia, a new inborn errors of metabolism. *Pediatr Res* 1968; **2**: 519.
- Lehnert W, Sperl W, Suormala T, Baumgartner ER. Propionic acidemia: clinical, biochemical and therapeutic aspects (experience in 30 patients). *Eur J Pediatr* 1994; **153** (7 Suppl. 1): S68–80.
- Nyhan WL, Sakati NA. Propionic acidemia. In: Nyhan WL, Sakati NA (eds). *Diagnostic Recognition of Genetic Disease*. Philadelphia, PA: Lea & Febiger, 1987: 36–41.
- Wolf B, Hsia YE, Sweetman L *et al.* Propionic acidemia: a clinical update. *J Pediatr* 1981; **99**: 835.
- North KN, Korson MS, Gopal YR *et al.* Neonatal-onset propionic acidemia. Neurologic and developmental profiles, and implications for management. *J Pediatr* 1995; **126**: 916.
- Surtees RAH, Matthews EE, Leonard JV. Neurologic outcome of propionic acidemia. *Ped Neurol* 1992; **8**: 333.
- Brandt IK, Hsia YE, Clement DH, Provence SA. Propionic acidemia (ketotic hyperglycinemia): dietary treatment results in normal growth and development. *Pediatrics* 1974; **53**: 391.
- Nyhan W, Bay C, Webb E *et al.* Neurologic nonmetabolic presentation of propionic acidemia. *Arch Neurol* 1999; **56**: 1143.
- Ozand PT, Rashed M, Gascon GG *et al.* Unusual presentations of propionic acidemia. *Brain Dev* 1994; **16**(Suppl.): 46.
- Sethi KD, Ray R, Roesel RA *et al.* Adult-onset chorea and dementia with propionic acidemia. *Neurology* 1992; **39**: 1343.
- Haas RH, Marsden DL, Capistrano-Estrado S *et al.* Acute basal ganglia infarction in propionic acidemia. *J Child Neurol* 1995; **10**: 18.
- Hamilton RL, Haas RH, Nyhan WL *et al.* Neuropathology of propionic acidemia: a report of two patients with basal ganglia lesions. *J Child Neurol* 1995; **10**: 25.
- Burlina AP, Baracchini C, Carollo C, Burlina AB. Propionic acidemia with basal ganglia stroke: treatment of acute extrapyramidal symptoms with L-DOPA. *J Inher Metab Dis* 2001; **24**: 596.
- Sweetman L, Nyhan WL, Cravens J *et al.* Propionic acidemia presenting with pancytopenia in infancy. *J Inher Metab Dis* 1979; **2**: 65.
- Yu A, Sweetman L, Nyhan WL. The pathogenetic mechanism of recurrent mucocutaneous candidiasis in a patient with methylmalonic acidemia (MMA). *Clin Res* 1981; **29**: 124A.
- Muller S, Falkenberg N, Monch E, Jakobs C. Propionic acidemia and immune deficiency. *Lancet* 1980; **1**: 551.
- Wolf B, Paulsen EP, Hsia YE. Asymptomatic propionyl CoA carboxylase deficiency in a 13-year-old girl. *J Pediatr* 1979; **95**: 563.

30. van der Meer B, Poggi F, Spada M. Clinical outcome and long-term management of 17 patients with propionic acidemia. *Eur J Pediatr* 1996; **155**: 205.
31. Harding BD, Leonard JV, Erdohazi M. Propionic acidemia: a neuropathological study of two patients presenting in infancy. *Neuropathol Appl Neurobiol* 1991; **17**: 133.
32. Nyhan WL, Chisolm JJ, Edwards RO. Idiopathic hyperglycinemia. III. Report of a second case. *J Pediatr* 1963; **62**: 540.
33. Lee TM, Addonizio LJ, Barshop BA, Chung WK. Unusual presentation of propionic acidemia isolated cardiomyopathy. *J Inherit Metab Dis* 2009; **154**. Available from: www.mendeley.com/research/unusual-presentation-of-propionic-acidemia-as-isolated-cardiomyopathy/.
34. Mardach R, Verity MA, Cederbaum SD. Clinical, pathological, and biochemical studies in a patient with propionic acidemia and fatal cardiomyopathy. *Mol Genet Metab* 2005; **85**: 286.
35. Baumgartner D, Scholl-Bürgi S, Sass J et al. Prolonged QTc intervals and decreased left ventricular contractility in patients with propionic acidemia. *J Pediatr* 2007; **150**: 192.
36. Hsia YE, Scully KJ, Rosenberg LE. Human propionyl CoA carboxylase: some properties of the partially purified enzyme in fibroblasts from controls and patients with propionic acidemia. *Pediatr Res* 1979; **13**: 746.
37. Wolf B, Hsia YE, Rosenberg LE. Biochemical differences between mutant propionyl-CoA carboxylases from two complementation groups. *Am J Hum Genet* 1978; **30**: 455.
38. Wolf B, Rosenberg LE. Heterozygote expression in propionyl coenzyme A carboxylase deficiency: differences between major complementation groups. *J Clin Invest* 1978; **62**: 931.
39. Saunders M, Sweetman L, Robinson B et al. Biotin-responsive organic aciduria: multiple carboxylase defects and complementation studies with propionic acidemia in cultured fibroblasts. *J Clin Invest* 1979; **64**: 1695.
40. Gravel RA, Lam KF, Scully KJ, Hsia YE. Genetic complementation of propionyl-CoA carboxylase deficiency in cultured human fibroblasts. *Am J Hum Genet* 1977; **29**: 378.
41. Wolf B, Willard HF, Rosenberg LE. Kinetic analysis of genetic complementation in heterokaryons of propionyl-CoA carboxylase-deficient human fibroblasts. *Am J Hum Genet* 1980; **32**: 16.
42. Lamhonwah AM, Lam KF, Tsui F et al. Assignment of the α and β chains of human propionyl-CoA carboxylase to genetic complementation groups. *Am J Hum Genet* 1983; **35**: 889.
43. Lamhonwah AM, Gravel RA. Propionic-acidemia: absence of alpha chain mRNA in fibroblasts from patients of the pccA complementation group. *Am J Hum Genet* 1987; **41**: 1124.
44. Kalousek F, Orsulak MD, Rosenberg LE. Absence of cross reacting material in isolated propionyl CoA carboxylase deficiency: nature of residual carboxylating activity. *Am J Hum Genet* 1983; **35**: 409.
45. Ohura T, Kraus JP, Rosenberg LE. Unequal synthesis and differential degradation of propionyl-CoA carboxylase subunits in cells from normal and propionic acidemia patients. *Am J Hum Genet* 1989; **45**: 33.
46. Lamhonwah AM, Mahuran D, Gravel RA. Human mitochondrial propionyl-CoA carboxylase: localization of the N-terminus of the pro- and mature α chains in the deduced primary sequence of a full length cDNA. *Nucleic Acids Res* 1989; **17**: 4396.
47. Stankovics J, Ledley FD. Cloning of functional alpha propionyl-CoA carboxylase and correction of enzyme deficiency in pccA fibroblasts. *Am J Hum Genet* 1993; **52**: 144.
48. Ohura T, Ogasawara M, Ikeda H et al. The molecular defect in propionic acidemia: exon skipping cause by an 8-bp deletion from an intron in the PCCB allele. *Hum Genet* 1993; **92**: 397.
49. Kennerknecht I, Suormala T, Barbi G, Baumgartner ER. The gene coding for the alpha-chain of human propionyl-CoA carboxylase maps to chromosome band 13q32. *Hum Genet* 1990; **86**: 238.
50. Kraus JP, Williamson CL, Firgaira FA et al. Cloning and screening with nanogram amounts of immunopurified mRNAs: cDNA cloning and chromosomal mapping of cystathionine beta-synthase and the beta subunit of propionyl-CoA carboxylase. *Proc Natl Acad Sci USA* 1986; **83**: 2047.
51. Tahara T, Kraus JP, Rosenberg LE. An unusual insertion/deletion in the gene encoding the beta-subunit of propionyl-CoA carboxylase is a frequent mutation in Caucasian propionic acidemia. *Proc Natl Acad Sci USA* 1990; **87**: 1372.
52. Tahara T, Kraus JP, Ohura T et al. Three independent mutations in the same exon of the PCCB gene: differences between Caucasian and Japanese propionic acidemia. *J Inherit Metab Dis* 1993; **16**: 353.
53. Ohura T, Miyabashi S, Narisawa K, Tada K. Genetic heterogeneity of propionic acidemia: analysis of Japanese patients. *Hum Genet* 1991; **87**: 41.
54. Campeau E, Dupuis L, Leclerc D, Gravel RA. Detection of a normally rare transcript in propionic acidemia patients with mRNA destabilizing mutations in the PCCA gene. *Hum Mol Genet* 1999; **8**: 107.
55. Campeau E, Dupuis L, Leon-del-Rio A, Gravel R. Coding sequence mutations in the alpha subunit of propionyl-CoA carboxylase in patients with propionic acidemia. *Mol Genet Metab* 1999; **67**: 1.
56. Leon-del-Rio A, Gravel RA. Sequence requirements for the biotinylation of carboxyl-terminal fragments of human propionyl-CoA carboxylase alpha subunit expressed in *Escherichia coli*. *J Biol Chem* 1994; **269**: 22964.
57. Hoenicka J, Muro J, Rodriguez-Pombo P et al. Prevalence of the novel mutation A497V in the PCCB gene in Spanish propionic acidemia patients from a small village. *Med Genet* 1997; **9**: 4311.
58. Gompertz D, Goodey PA, Thom H et al. Prenatal diagnosis and family studies in case of propionic acidemia. *Clin Genet* 1975; **8**: 244.
59. Sweetman FR, Gibson KM, Sweetman L, Nyhan WL. Activity of biotin-dependent and GABA metabolizing enzymes in chorionic villus samples: potential for 1st trimester prenatal diagnosis. *Prenat Diagn* 1986; **6**: 187.
60. Willard HF, Ambani LM, Hart AC et al. Rapid prenatal and postnatal detection of inborn errors of propionate, methylmalonate, and cobalamin metabolism: A sensitive assay using cultured cells. *Hum Genet* 1976; **34**: 277.
61. Naylor G, Sweetman L, Nyhan WL et al. Isotope dilution analysis of methylcitric acid in amniotic fluid for the prenatal

- diagnosis of propionic and methylmalonic acidemia. *Clin Chim Acta* 1980; **107**: 175.
62. Buchanan PD, Kahler SG, Sweetman L, Nyhan WL. Pitfalls in the prenatal diagnosis of propionic acidemia. *Clin Genet* 1980; **18**: 177.
 63. Hillman RE, Sowers LH, Cohen JL. Inhibition of glycine oxidation in cultured fibroblasts by isoleucine. *Pediatr Res* 1973; **7**: 945.
 64. Wadlington WB, Kilroy A, Ando T *et al*. Hyperglycinemia and propionyl CoA carboxylase deficiency and episodic severe illness without consistent ketosis. *J Pediatr* 1975; **86**: 707.
 65. Ando T, Rasmussen K, Wright M, Nyhan WL. Isolation and identification of methylcitrate, a major metabolic product of propionate in patients with propionic acidemia. *J Biol Chem* 1972; **247**: 2200.
 66. Wendel U, Eissler A, Sperl W, Schadewaldt P. On the differences between urinary metabolite excretion and odd-numbered fatty acid production in propionic and methylmalonic acidemias. *J Inherit Metab Dis* 1995; **18**: 584.
 67. Van Gennip AH, Van Lenthe H, Abeling NGGM *et al*. Inhibition of β -ureidopropionase by propionate may contribute to neurological complications in patients with propionic acidemia. *J Inherit Metab Dis* 1997; **20**: 379.
 68. Kolker S, Okun JG, Horster F *et al*. 3-Ureidopropionate contributes to the neuropathology of 3-ureidopropionase deficiency and severe propionic aciduria: a hypothesis. *J Neurosci Res* 2001; **66**: 666.
 69. Stumpf DA, McAfee J, Parks JK, Euren L. Propionate inhibition of succinate: CoA ligase (GDP) and the citric acid cycle in mitochondria. *Pediatr Res* 1980; **14**: 1127.
 70. Van Hove JLK, Chace DH, Kahler SG, Millington DS. Acylcarnitines in amniotic fluid: application to the prenatal diagnosis of propionic acidemia. *J Inherit Metab Dis* 1993; **16**: 361.
 71. Schwab MA, Sauer SW, Okun JG *et al*. Secondary mitochondrial dysfunction in propionic aciduria: a pathogenic role for endogenous mitochondrial toxins. *Biochem J* 2006; **398**: 107.
 72. Coude FX, Sweetman L, Nyhan WL. Inhibition by propionyl CoA of N-acetylglutamate synthetase in rat liver mitochondria. *J Clin Invest* 1979; **64**: 1544.
 73. Barnes ND, Hull D, Balgobin L, Gompertz D. Biotin-responsive propionic acidemia. *Lancet* 1970; **2**: 244.
 74. Barshop BA, Yoshida I, Ajami A *et al*. Metabolism of 1-¹³C-propionate *in vivo* in patients with disorders of propionate metabolism. *Pediatr Res* 1991; **30**: 15.
 75. Nyhan WL, Fawcett N, Ando T *et al*. Response to dietary therapy in B₁₂ unresponsive methylmalonic acidemia. *Pediatrics* 1973; **51**: 539.
 76. Ney DN, Bay C, Saudubray JM *et al*. An evaluation of protein requirements in methylmalonic acidemia. *J Inherit Metab Dis* 1985; **8**: 132.
 77. Nyhan WL. Disorders of propionate metabolism. In: Bickel H, Wachtel U (eds). *Inherited Diseases of Amino Acid Metabolism. Recent Progress in the Understanding, Recognition and Management*. International Symposium on Heidelberg, 1984. Stuttgart: Georg Thiem Verlag Thieme, 1985: 363–82.
 78. Saudubray JM. Use of new diagnostic technology in the management of inborn errors of metabolism, in *Proceedings of the VI International Congress of Inborn Errors of Metabolism* (Milan, Italy), 1994: 28.
 79. Wendel U, Baumgartner RE, Van Der Meer SB, Spaapen LJM. Accumulation of odd-numbered long chain fatty acids in fetuses and neonates with inherited disorders of propionate metabolism. *Pediatr Res* 1991; **29**: 403.
 80. Roe CR, Bohan TP. L-carnitine therapy in propionic acidemia. *Lancet* 1982; **1**: 1411.
 81. Chalmers RA, Roe CR, Stacey TE, Hoppel CL. Urinary excretion of L-carnitine and acylcarnitines by patients with disorders of organic acid metabolism: evidence for secondary insufficiency of L-carnitine. *Pediatr Res* 1984; **18**: 1325.
 82. Roe CR, Hoppel CL, Stacey TE *et al*. Metabolic response to carnitine in methylmalonic aciduria: an effective strategy for elimination of propionyl groups. *Arch Dis Child* 1983; **58**: 916.
 83. Roe CR, Millington DS, Maltby DA *et al*. L-carnitine enhances excretion of propionyl coenzyme A as propionyl carnitine in propionic acidemia. *J Clin Invest* 1984; **73**: 1785.
 84. Wolf JA, Thuy LP, Haas R *et al*. Carnitine reduces fasting ketogenesis in patients with disorders of propionate metabolism. *Lancet* 1986; **1**: 289.
 85. Marsden D, Barshop BA, Capistrano-Estrada S *et al*. Anabolic effect of human growth hormone: management of inherited disorders of catabolic pathways. *Biochem Med Metab Biol* 1994; **52**: 145.
 86. Thompson GN, Chalmers RA. Increased urinary metabolite excretion during fasting in disorders of propionate metabolism. *Pediatr Res* 1990; **27**: 413.
 87. Thompson GN, Walter JH, Bresson JL *et al*. Sources of propionate in inborn errors of metabolism. *Metabolism* 1990; **39**: 1133.
 88. Picca S, Dionisi-Vici C, Abeni D *et al*. Extracorporeal dialysis in neonatal hyperammonemia: modalities and prognostic indicators. *Pediatr Nephrol* 2001; **16**: 862.
 89. Gebhardt B, Dittrich S, Vlaho S. Carbamylglutamate protects patients with decompensated propionic aciduria from hyperammonemia. *J Inherit Metab Dis* 2005; **28**: 241.
 90. Schwahn BC, Pieterse L, Bisset WM *et al*. Biochemical efficacy of N-carbamylglutamate in neonatal severe hyperammonemia due to propionic acidemia. *Eur J Pediatr* 2010; **169**: 133.
 91. Nyhan WL, Rice-Asaro M, Acosta P. Advances in the treatment of amino acid and organic acid disorders. In: Desnick RJ (ed.). *Treatment of Genetic Diseases*. New York: Churchill Livingstone, 1991: 45–67.
 92. Kalloghlian A, Gleispach H, Ozand PT. A patient with propionic acidemia managed with continuous insulin infusion and total parenteral nutrition. *J Child Neurol* 1992; **7**(Suppl.): S88.
 93. Thompson GN, Chalmers RA, Walter JH *et al*. The use of metronidazole in management of methylmalonic and propionic acidemias. *Eur J Pediatr* 1990; **149**: 792.
 94. Leonard JV, Walter JH, McKiernan PJ. The management of organic acidemias: the role of transplantation. *J Inherit Metab Dis* 2001; **24**: 309.
 95. Saudubray JM, Touati G, Delonlay P *et al*. Liver transplantation in propionic acidemia. *Eur J Pediatr* 1999; **158**: 65–9.

Methylmalonic acidemia

Introduction	19	Treatment	27
Clinical abnormalities	20	References	28
Genetics and pathogenesis	24		

MAJOR PHENOTYPIC EXPRESSION

Recurrent episodes of ketosis, acidosis, vomiting, and dehydration; anorexia, failure to thrive; hepatomegaly; osteoporosis; neutropenia; thrombocytopenia; hyperglycinemia; elevated concentrations of methylmalonic acid (MMA) in blood and urine; and defective activity of methylmalonyl CoA mutase.

INTRODUCTION

Methylmalonic acidemia represents a family of disorders of the metabolism of branched-chain amino acids in which the activity of methylmalonyl CoA mutase is defective (Figure 3.1). Patients with the inborn error of metabolism were first reported in 1967 by Oberholzer *et al.* [1] and by Stokke *et al.* [2]. In 1968, Rosenberg and colleagues [3] first clearly distinguished these patients from those with propionic acidemia (Chapter 2), in whom the clinical presentation is often virtually identical.

Genetic heterogeneity was evident early in the demonstration in that some patients with methylmalonic acidemia were responsive to large doses of vitamin B₁₂, while some others were not [4]. The methylmalonyl CoA mutase enzyme has a vitamin B₁₂-derived cofactor, adenosylcobalamin AdoCbl. Patients who are B₁₂-responsive clinically have defects in the synthesis of the cofactor. Unresponsive patients have defects in the apoenzyme itself. Complementation studies have indicated the presence of distinct groups (Figure 3.1). Those with apoenzyme defects have been designated mut⁻ or mut⁰ depending on whether they have little or no residual mutase activity. Groups A and B represent defects in AboCbl synthesis. A differential diagnosis of methylmalonic acidemia is shown in Table 3.1.

The cobalamin (Cbl) C and D represent a different type of disorder in which methylmalonic acidemia accompanies elevated concentrations of homocysteine and cystathionine in blood and urine (Chapter 4) [5]. In

Table 3.1 Different types of methylmalonic acidemia

Methylmalonyl CoA mutase deficiency (mut ⁰ , mut ⁻)
Adenosyltransferase deficiency (Cbl B)
Cbl A
Homocystinuria with methylmalonic acidemia (MMA) (Cbl C, D) (Chapter 4)
Methylmalonyl CoA epimerase deficiency
B ₁₂ deficiency (vegan mother – breast feeding) (vegan child)
Pernicious anemia (intrinsic factor deficiency)
Methylmalonyl CoA epimerase deficiency
Transcobalamin II deficiency
B ₁₂ transport from lysosome defect (Cbl F)
Succinyl CoA ligase deficiency (SUCLG1, SUCLA2)

these groups, defective remethylation of homocysteine to methionine is the consequence of a failure to transform B₁₂ to either of the coenzymatically active derivatives, adenosylcobalamin or methylcobalamin. Cobalamin F disease reflects abnormalities in the transport of cobalamin out of lysosomes, analogous to the defect that causes cystinosis (Chapter 71). Methylmalonic aciduria is also seen in acquired deficiency of B₁₂ [6], in pernicious anemia and in transcobalamin II deficiency [7]. In B₁₂ deficiency and in intrinsic factor deficiency, the excretion of methylmalonic acid in the urine is a more reliable index of depletion of body stores of cobalamin than the blood level of B₁₂.

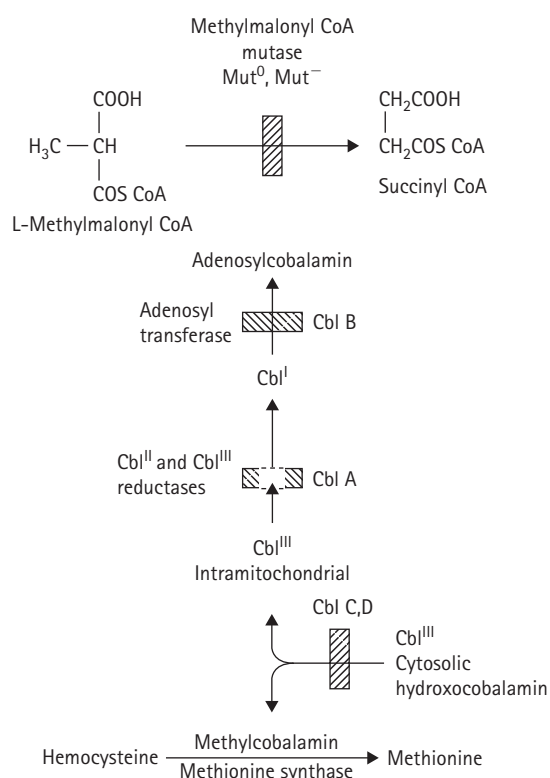


Figure 3.1 MethylmalonylCoA mutase, the site of the defect in methylmalonic aciduria is shown under its cofactor adenosylcobalamin (AdoCbl) the cofactor for the mutase enzyme. Cobalamin cofactor synthesis is also illustrated in the formation of AdoCbl. The sites of the defects in the various complementation groups identified are shown as Cbl A, B, and D. Apoenzyme defects are referred to as Mut⁰ or Mut⁻.

Methylmalonic acidemia resulting from a defect in the methylmalonyl, CoA epimerase enzyme, long suspected to exist has now been documented by mutational analysis in a homozygous patient [8, 9]. Methylmalonic aciduria of modest degree has been the clue to a metabolic diagnosis in patients with succinyl CoA ligase (synthase) deficiency which leads to a mitochondrial DNA depletion syndrome [10–13]. This complex has two subunits, α and β coded from by genes *SUCLA2* and *SUCLG1*. Mutations in both have been documented [11, 13].

All of the methylmalonic acidemias reflect defective activity of methylmalonyl CoA mutase [14]. In inherited defects of the apoenzyme and in abnormalities in coenzyme synthesis, the enzymatic mutase abnormality is evident in tissues, leukocytes, and cultured fibroblasts. The mutase gene has been cloned [15] and mapped to chromosome 6 [16]. Some 200 mutations in the mutase gene have been documented [17–19].

CLINICAL ABNORMALITIES

Patients with methylmalonic acidemia usually present first with a typical organic acidemia picture of overwhelming

illness very early in life [1–3, 20–23]. A majority of the reported patients, especially those with apoenzyme defects, have died in such an episode. We believe that prior to newborn screening, many patients died with the disease unrecognized, and that the disease is more common than previously realized. A typical episode is ushered in with ketonuria and vomiting, followed by acidosis, dehydration, and lethargy, leading, in the absence of aggressive treatment, to coma and death.

Episodes of acute illness are recurrent. They may follow even minor infections. Furthermore, patients are unusually prone to infection. Episodes are also a consequence of feeding; these patients are intolerant of the usual quantities of dietary protein. More specifically, they are intolerant to the amino acids isoleucine, valine, threonine, and methionine, all of which are catabolized through the pathway of propionate and methylmalonate metabolism (see Figure 2.1). Episodic disease may follow a pattern in which the patient is admitted to hospital *in extremis*, treated vigorously with parenteral fluid and electrolytes, which leads to recovery; oral feedings are reintroduced and, following a sufficient time of ingestion of the usual dietary amounts of protein, another episode of crisis supervenes, and in one of these episodes the patient dies.

During episodes of ketosis, acidosis may be extreme. Arterial pH values as low as 6.9 have been recorded, and the serum bicarbonate is often 5 mEq/L or less. Ketosis is massive. Hypoglycemia has been observed and has led to seizures during acute episodes. Elevated concentrations of glycine in the blood and urine may be striking, and this may be an early clue to the diagnosis. Concentrations of glycine as high as 1500 μ mol/L have been observed in the plasma. However, concentrations of glycine may also be normal, even in the same patient. Hyperammonemia may complicate the initial episode in which levels may be as high as in urea cycle defects and lead to deep coma and apnea [24, 25]. With development, this propensity to hyperammonemia is lost, and acute episodes after the first year are seldom complicated by hyperammonemia [24]. The typical acute episode of disease with massive ketosis has led us to examine the urine for ketones in any acidotic infant. In addition, in management we provide ketostix to parents and instruct them to test for ketones at any sign of illness, especially infections; on the other hand, this too can be misleading. We are currently following an adult with mut⁰ methylmalonic acidemia who develops acute episodes of acidosis with no ketonuria. Kussmaul breathing is still an alerting feature.

Failure to thrive may be the initial presentation in this disease and failure of linear growth may be striking (Figures 3.2, 3.3, and 3.4). Developmental failure may parallel the inability to increase weight, height, and head circumference. Anorexia is severe, and usually requires tube feeding (Figure 3.3). In addition to the fact that the ketoacidotic episode is often ushered in with vomiting, these patients vomit frequently in infancy, and this may contribute to failure to thrive.



Figure 3.2 LG: A 14-month-old girl with methylmalonic acidemia. The size, that of a three-month-old infant, reflects the severe failure to thrive characteristic of this disorder. The frog-leg position illustrates the marked hypotonia.



Figure 3.4 This infant with methylmalonic acidemia had also failed to thrive and was anorexic. She also had alopecia.



Figure 3.3 ME: A 13-month-old boy with methylmalonyl CoA mutase deficiency who also failed to gain in weight, height, or head circumference since three months of age. The nasogastric tube is typical, indicating the extreme anorexia. The bright red erythematous lesions are the characteristic monilial infection of the infant in poor metabolic control.

A variety of skin lesions may be seen (Figure 3.5). Most often, this is a manifestation of moniliasis. Mucocutaneous moniliasis may also be reflected in cracking and erythema at the angles of the mouth and the eyes (Figure 3.3) [26].



Figure 3.5 The same infant had a flord perineal dermatitis.

Patients with methylmalonic acidemia have a striking resemblance to each other, especially in infancy (Figure 3.6). The characteristic face includes a high forehead, broad nasal bridge, epicanthal folds, a long smooth filtrum, and a triangular mouth. A few have had other minor anomalies [23]. A recent patient of ours had inverted nipples. One patient had multiple defects at birth, including cardiac septal defects, hydronephrosis, and an appearance of Sotos syndrome [27].

Neurological manifestations of methylmalonic acidemia are varied. In infancy and childhood, these features appear to be more consequences of the physiology of the acute episode of shock and diminished cerebral perfusion or hypoglycemia, and especially hyperammonemia with or without cerebral edema, than the metabolic abnormality itself [28]. Developmental impairment is evident in most patients in infancy, but in some this may be more



Figure 3.6 (A, B, C, D) Composite picture of four patients with methylmalonic acidemia highlighting the similarity of the facial features. A high forehead, broad nasal bridge and wide-appearing eyes with epicanthal folds and a long smooth filtrum were characteristic. In some, the nose was upturned and in some, the mouth was triangular. The patient in panel (C) had had a preauricular skin tag which was removed.

apparent than real; evidence of severe chronic disease and extreme hypotonia, both of which interfere with motor development. Catch up has been observed in patients successfully treated, and the IQ may be normal [23, 28]. The impairment and disability phenotype of 33 patients with isolated methylmalonic acidemia has been set out [29]. Seventeen had lesions in the globus pallidus on magnetic resonance imaging (MRI). Neurological findings, including ataxia, dystonia, dyskinesia, dysarthria, chorea, clonus, extrapyramidal signs, or tremors, were found in a majority (25 out of 33). Impaired balance and coordination were common; almost half had difficulties with bathing or dressing. Educational support was commonly provided.

Neurological abnormality is more common in

patients with apoenzyme defects than those with defects in cobalamin synthesis [30–32]; however, abnormalities in central nervous system function may be seen in any patient with methylmalonic acidemia (Figures 3.7, 3.8, and 3.9). Dystonia and weakness profound enough to lead to a wheelchair-bound state has been observed in methylmalonic acidemia [33]. This has been associated with neuroradiologic evidence of abnormality in the basal ganglia, which has frequently been encountered in disorders of propionate metabolism [34–36]. In patients imaged by computed tomography (CT) or MRI, specific lesions are regularly seen in the basal ganglia (Figures 3.8 and 3.9), even in patients with no relevant clinical findings. Lesions in the globus pallidus are regularly seen in mut^0 and mut^-

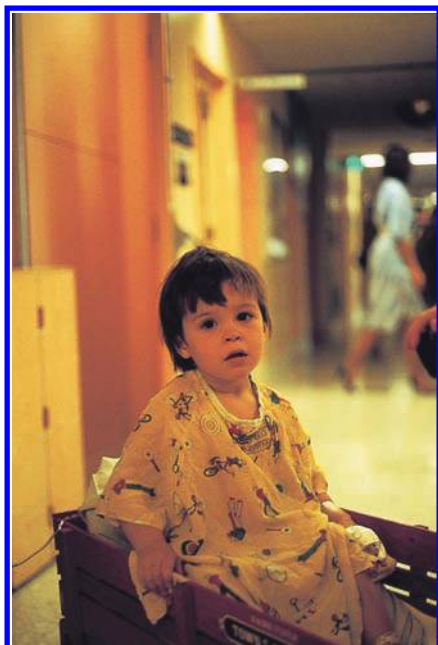


Figure 3.7 TJ: A boy with B_{12} -responsive methylmalonic acidemia of the cbl A type. He had only the initial severe acidotic episode, but his behavior was sufficiently unusual that he had been characterized as autistic.



Figure 3.8 Magnetic resonance image of the brain of TJ revealed increased intensity of T_2 signal in the basal ganglia.

patients, but they are also seen in cobalamin-responsive patients [34–40]. At the extreme, a syndrome of metabolic stroke has been reported with what looks like infarction of the basal ganglia, especially the globus pallidus, and acute dystonia [33, 35, 41]. Diffusion-weighted imaging (DW-MRI) has been reported [42] to aid in the detection of acute basal ganglia infarction and to aid in the distinction of acute from chronic changes. The authors emphasized that basal ganglia stroke can occur even after many years without acute metabolic imbalance. Decreased white matter attenuation on CT and high T_2 signal on MRI may be seen

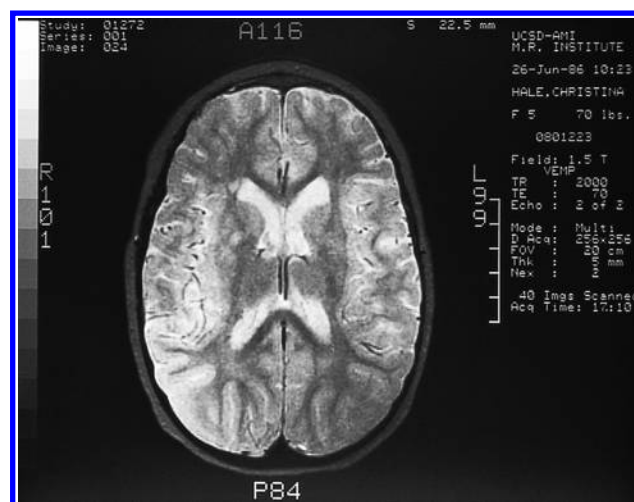


Figure 3.9 Magnetic resonance image of CH, a patient with methylmalonyl CoA mutase deficiency. There was a diffuse pattern of abnormal signal intensity in the cerebral hemispheres and focal areas of abnormal signal in the basal ganglia.

early [32, 39, 43]. This may progress to cerebral atrophy and quadriplegia [33]. Harting and colleagues [44] have called attention to a spectrum of changes in the MRI of patients with methylmalonic, in addition to the characteristic lesions of the basal ganglia (pallidum). Their patients had delayed maturation of the brain, immature gyral patterns, incomplete operculum, and white matter disorder. Changes were found in the brain stem and cerebellum in all of their children, as well as T_2 hyperintensity and volume loss. Some patients have had convulsions, and abnormalities of the electroencephalogram (EEG) are more common [21, 33]. In one patient, who died at 6 days of age with hyperammonemic coma and ketoacidosis, there was a burst suppression pattern [45]. Neuropathology in this patient revealed diffuse gliosis in the white matter, Alzheimer type II cells, and cerebellar hemorrhage.

In some patients, abnormal neurologic signs increased with age [32, 33]. Among patients surviving longer, late effects, including central nervous system abnormalities are becoming apparent [32]. We have reported a mut^0 young adult who developed weakness in her teens and became wheelchair-bound. She had acute involuntary spasms of the legs and more general spasms resembling myocymia. Cognitive function was not impaired. Among late effects, blindness developed in a 21-year-old patient two months before he died; he had been in a wheelchair but rode horses and could sail a yacht and drive farm equipment before developing optic atrophy [46]. Optic atrophy is becoming increasingly recognized as a late effect of this disease (Bey *et al.*, personal observations).

Some patients have hepatomegaly. Liver function tests are normal. Renal functional impairment has been reported [1] and we have observed chronic renal tubular acidosis [47]. Hyperuricemia is usually present, a consequence of competition for its renal tubular excretion.

Urate nephropathy and renal failure have been reported [48]. Tubulointerstitial nephritis has been reported in four biopsied patients of 15 reported with renal disease [49]. End-stage renal disease requiring dialysis and/or transplantation has been observed, as another late complication [35, 50, 51].

Pancreatitis has been reported in a variety of organic acidemias [52]. Among the patients, methylmalonic acidemia was particularly prominent. Five of nine patients had methylmalonic acidemia; of these, two died. One of our adult patients with *mut*⁰ disease died of acute hemorrhagic pancreatitis.

Transient thrombocytopenia has been observed in infancy. Neutropenia is a regular occurrence except in the case of successful treatment and reduction in the accumulation of methylmalonic acid in body fluids. Anemia may occur, especially in the first month of life. Recurrent infections are common.

Chronic moniliasis is highly relevant to metabolic control. High levels of methylmalonate and other intermediates that accumulate when patients are out of control inhibit the maturation of hematopoietic cells and also of T cells, so the T-cell number is low. The response of T cells to *Candida* is also specifically altered when levels are high [26]. When metabolite levels are lowered by treatment, skin lesions disappear and T-cell responsiveness to *Candida* returns. Osteoporosis has been found regularly and we have observed femoral and tibial fractures.

Patients with *B*₁₂ responsiveness, in both the CblA and CblB complementation groups not only had milder disease, they presented later than those with *mut*⁰ or *mut*⁻ disease [30, 53]. Some 80 percent of *mut*⁰ patients presented within the first week of life; 42 and 33 percent, respectively, of CblA and CblB patients presented this early. The *mut*⁰ patients were predominantly dead or severely impaired at follow up. Most died within two months of diagnosis. Most of the CblA and CblB patients were alive at follow up.

At least four successful pregnancies have been reported in women with methylmalonic acidemia [54, 55] despite evidence of renal impairment. One was *mut*⁻ and one *B*₁₂-responsive. As predicted, levels of MMA decreased dramatically as the fetus grew [55]. These experiences documented that MMA is not teratogenic.

Some patients with MMA have been clinically normal. Presumably, these individuals are *mut*⁻ variants with a considerable level of activity *in vivo*. So-called 'benign methylmalonic acidemia' has been reported in at least nine clinically normal individuals [56, 57], eight of them identified through routine neonatal screening [56]. Some of these patients may excrete quite large amounts of MMA. In the Quebec program of screening neonatal urine for MMA [57], a follow-up study of 122 individuals with MMA excretion over 1400 mmol/mol creatinine indicated that MMA excretion had resolved by one year of age in 65 and in ten more over 15 months to seven years; so a majority were transient. The rest were 13 symptomatic and 22 asymptomatic. MMA levels in blood and urine were

appreciably higher in the symptomatic patients. Careful study of the asymptomatic patients revealed one to be *mut*⁻ and the rest undiagnosed. All of the asymptomatic patients were found to be clinically and cognitively normal at follow up. Programs of neonatal screening are finding patients with MMA in appreciably greater numbers than were evident from experience with illness presentations. In the California pilot study of screening by tandem mass spectrometry (MS/MS), a newborn population screening of 309,074 yielded eight methylmalonic acidemic patients. This prevalence rate of 1 in 3800 represented the third most common disorder detected and the only prevalent organic acidemia. Some of these patients might represent those who might have died undiagnosed, but some of them are likely to represent more benign disease. Clues from the Quebec study [57] indicated that the benign patients are likely to have no urinary metabolites of propionate, such as hydroxypropionate or methylcitrate, and many excrete malonic acid in amounts of 60–227 mmol/mol creatinine.

In addition to the differential diagnosis of methylmalonic acidemia shown in Table 3.1, there are a number of patients of variable, atypical phenotype in whom the molecular nature of the disease has not been defined [58, 59]. Most have had appreciably lower levels of MMA than in a classic patient and activities of the mutase enzyme are normal. Treatment with *B*₁₂ had no effect, and protein restriction may not decrease the excretion of MMA. Some, but not all have also excreted malonic acid. These patients have not had a crisis of ketoacidotic metabolic imbalance. They have usually been investigated because of failure to thrive or developmental delay. Some have had athetoid movements, myopathy, ophthalmoplegia or pyramidal tract signs [58]. Two siblings developed renal tubular acidosis with hypercalciuria, one of whom developed nephrocalcinosis [59].

GENETICS AND PATHOGENESIS

Each of the forms of methylmalonic acidemia is determined by a rare autosomal recessive gene. Complementation studies [60, 61] have indicated that there are at least four distinct forms of methylmalonic acidemia. Furthermore, the *mut* apoenzyme defect group is heterogeneous. *Mut*⁰ patients have no activity of the enzyme, while *mut*⁻ patients have a spectrum of residual activity. Heterozygote detection by enzyme analysis may be unreliable.

Prenatal detection of methylmalonic acidemia has been accomplished by assay of the activity of methylmalonyl CoA mutase in cultured amniotic cells [62]. The diagnosis has also been made chemically by assay of the maternal urine for methylmalonic acid [63], but this may not be reliable until quite late in pregnancy. Rapid, efficient chemical diagnosis can be made by direct analysis of the amniotic fluid for methylcitric acid or methylmalonic acid using stable isotope dilution methodology and gas chromatography-mass spectrometry (GCMS) [63–66].

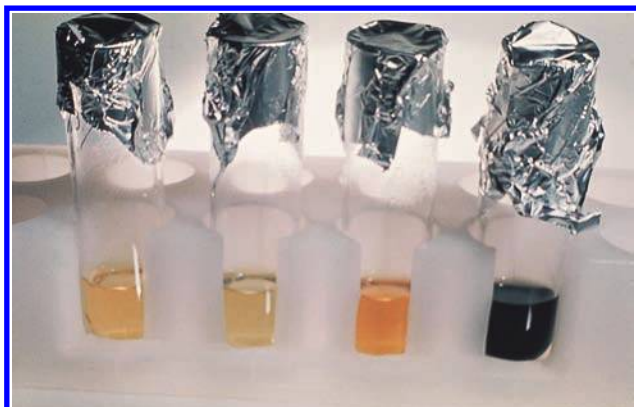


Figure 3.10 Colorimetric test of urine for methylmalonic acid. The dark green color which develops in the presence of p-nitroaniline has been used for screening.

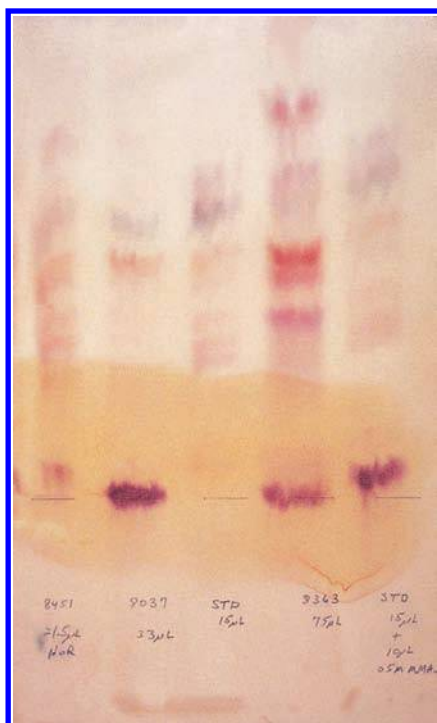


Figure 3.11 High voltage electrophoresis of the urine. The amino acids have been separated and stained with ninhydrin and then the paper was overstained with Fast Blue B giving a purple band at the origin in the presence of methylmalonic acid. Numbers 8037 and 8363 were patients with mut^0 methylmalonic acidemia.

Fully automated high throughput assay has been reported [67]. In a family in which the mutation is known, its determination can be used for prenatal diagnosis, and for heterozygote detection. An infant with defective adenosylcobalamin synthesis was diagnosed prenatally and effectively treated with cobalamin prenatally [68].

The diagnosis of methylmalonic acidemia is most readily made by assay of the urine for MMA (Figures 3.10

Table 3.2 Excretion of methylmalonic acid

Clinical status	Amount excreted (mmol/mol creatinine)
Normal	0–2
Mut^0 ; presentation	3000–13000
Mut^0 ; steady-state	200–2000
B_{12} -responsive; presentation	2000
B_{12} -responsive; steady-state	90–300
B_{12} -deficient infant	4500–5700
Transcobalamin II deficiency	600
Cobalamin C, D	270
Atypical-normal mutase	200
SuccinylCoA ligase	80–120
MethylmalonylCoA epimerase deficiency	50–300

and 3.11). Screening tests are now seldom used, and the diagnosis is usually made by organic acid analysis of the urine with GCMS. The amounts of methylmalonic acid excreted are enormous. Excretion of a gram a day by a tiny infant is not unusual. Normal individuals excrete less than 5 mg per 24 hours, amounts that are undetectable in the usual assays. A comparison of the amounts of methylmalonate excreted in patients with various forms of methylmalonic aciduria is shown in Table 3.2.

Methylmalonic acid, undetectable in the plasma of normal individuals, is present in patients in concentrations of 200–2500 $\mu\text{mol/L}$. The concentrations of methylmalonic acid in the cerebrospinal fluid (CSF) may equal that of the plasma. In patients with cobalamin deficiency, concentrations in CSF tend to be much higher than in plasma [69]. Propionic acid also accumulates in the plasma of patients with methylmalonic acidemia [70], and 3-hydroxypropionate [71] and methylcitrate [72] are found in the urine. The administration of isoleucine, threonine, valine, or methionine results in the formation of methylmalonic acid [73].

The diagnosis of methylmalonic acidemia is increasingly made by MS/MS, not only in programs of newborn screening, but by quantitative analysis of acylcarnitine profiles of plasma (Chapter 1). Answers are more rapidly available in an emergency situation than by GCMS of urinary methylmalonate. The quantification of urinary methylmalonate remains the best approach to monitoring the effectiveness of therapy. Electrospray MS/MS of urine in positive and negative modes has been reported as a rapid approach to diagnosis of a variety of inborn errors of metabolism [73]. Separation of succinic and methylmalonic acids was not achieved, but the disparity in amounts excreted in illness indicated utility in the diagnosis of methylmalonic acidemia. A liquid chromatography (LC)-MS/MS method with a deuterated

internal standard has been reported [74] that is rapid and accurate and correlated well with GCMS in the analysis of the same samples; it can be used for plasma or urine. Methodology for the analysis of MMA is of broad applicability in addition to the diagnosis and management of inborn errors of metabolism, because elevated plasma concentration of MMA that responds to B_{12} is the best indicator of tissue deficiency of cobalamin [75].

All patients with MMA have defective activity of methylmalonylCoA mutase (Figure 3.1), the enzyme that catalyzes the conversion of methylmalonylCoA to succinylCoA (see Figure 2.1). This enzyme lies on the direct degradative pathway for isoleucine, valine, threonine, and methionine. All of these amino acids have been shown to be major sources of methylmalonate in patients. On the other hand, lipids, although metabolizable via this pathway, do not contribute in measurable fashion to urinary methylmalonic acid [76].

Apoenzyme defect was first demonstrated in liver of four patients who died, by measuring the conversion of ^3H -methylmalonylCoA to ^3H -succinylCoA [14]. In the mut^0 group, mutase activity in cultured fibroblasts or tissues is undetectable even in the presence of adenosylcobalamin [77–79]. In the mut^- group, some residual activity is present. Heterogeneity has been demonstrated in the mut^0 group because some patients are cross-reactive material (CRM)-negative, and some have reduced amounts of CRM [80]. Some patients in the mut^- group may have much later and much milder clinical presentations. Enzyme activity of 2–75 percent of control was associated with CRM of 20–100 percent of control [80]. In studies of labeled enzyme synthesis, some mut^0 patients made unstable enzyme, which disappeared, while most made no detectable enzyme [81]. All mut^- patients made detectable newly synthesized enzyme.

The study of fibroblasts of patients with B_{12} -sensitive methylmalonic aciduria [82] clarified the nature of these disorders. The content of adenosylcobalamin is reduced, and the cells cannot convert ^{57}Co -hydroxycobalamin to ^{57}Co -adenosylcobalamin [83].

A simplified test for the overall enzymatic block at the mutase step is to test the conversion by cultured fibroblasts of ^{14}C -propionate to $^{14}\text{CO}_2$ [84]. Assessment of ^{14}C -MMA oxidation permits distinction of MMA from propionic acidemia. The extrapolation of this assay to the incorporation of ^{14}C -propionate into acid precipitable material has simplified the procedure [85]. This has been employed in studies of complementation among the inherited methylmalonic acidemias. Patients responsive to B_{12} were promptly subfractionated into two complementation groups, designated Cbl A and Cbl B [60, 61, 86, 87]. The Cbl A variants synthesize adenosylcobalamin normally from ^{57}Co -hydroxycobalamin and adenosine triphosphate (ATP) under reducing conditions [83]. In the Cbl B variants, adenosylcobalamin synthesis is defective under these conditions, and the defect has been shown to be in the adenosyltransferase [88, 89]. Patients with defects in

cobalamin synthesis generally present later than those with apoenzyme defects, and most survive the illness once diagnosed. Among the Cbl A patients, a clinical response to B_{12} is regularly seen, while in Cbl B patients only half respond to B_{12} with a decrease in the amounts of methylmalonic acid in body fluids, suggesting that there is a complete block in the unresponsive patients.

The cDNA for methylmalonyl CoA mutase was originally obtained from human cDNA hepatic libraries; it was used as a clone to localize the gene to human chromosome 6q12-21.2 [15, 16]. A highly informative restriction fragment length polymorphism (RFLP) at this locus, a *Hind*III polymorphism, has been used for heterozygote detection, prenatal diagnosis, and linkage analysis. By now, approximately 200 mutations have been identified in the *MUT* gene [17–19, 90, 91].

Four mutations in mut^- cells, all of which exhibited interallelic complementation, clustered near the carboxyl terminus of the protein. These missense mutations (R664W, G648D, G630E, and G626C) (respectively, arginine to tryptophan, and glycine to aspartic acid, glutamic acid, and cysteine) were close to another mutation (G717V) (glycine to valine) in the region that appears likely to be the cobalamin binding domain. The enzyme in these cells could be stimulated *in vitro* by very high concentrations of hydroxycobalamin. R694W was also found in the mut^- phenotype [18]. The enzyme specified by G717V mutation was shown to have a very high K_m for adenosylcobalamin. The enzyme bearing the G648D mutation also had a high K_m . It is of interest that six of seven mutations described in this area involved substitution for a glycine residue, suggesting altered secondary or tertiary structure [92]. Among mut^0 patients, mutations near the amino terminal of the protein eliminated enzyme activity entirely [93, 94]. G717V has been observed to be common in black Americans [92, 93]. Among Japanese, E117X was found in every patient reported [95]. At least one mutation has been reported, an N terminal deletion which interfered with processing of the enzyme, such that it was not taken up by the mitochondria [96]. Gene transfer has been employed [95] as a substitute for complementation in the distinction of mut from Cbl phenotypes. In 25 predominantly Spanish patients, frame shift mutations were prevalent [97]. Transfer of a normal mutase cDNA clone corrected activity as measured by ^{14}C -propionate assay. Transfer into Cbl fibroblasts had no effect on activity. In European patients with the CblA disease and a severe clinical phenotype, R145X was found in 43 percent of mutant alleles [98]. In two patients with the CblB disease, I96T and G97fs mutations were found [97]. A homozygous nonsense mutation R47X was found in a patient with mild methylmalonic aciduria in the epimerase gene (*MCEE*) [8]. In one group [97] of Cbl A patients, all mutations led to truncated proteins [97].

It may be relevant to pathogenesis that mitochondrial dysfunction has been observed in mut methylmalonic acidemia [99]. In *Mut* knockout mice, mega mitochondria

were found in hepatocytes early in life, and there was respiratory chain dysfunction with diminished cytochrome oxidase activity and low intracellular glutathione. Similar findings were observed in the liver of a patient who had undergone liver transplantation. The mice also developed tubulointerstitial renal disease. A patient with CblA disease responsive to B_{12} developed multiorgan failure and died; multiple defects in OXPHOS were found in the liver [100]. The terminal episode was ushered in by the sudden onset of optic atrophy.

TREATMENT

Patients with methylmalonic acidemia should first be tested for responsiveness to B_{12} . This is important for a majority of those responding have survived, while the majority of the unresponsive, who were detected as a result of ketoacidotic illness, have not survived [30] or have survived with major neurologic disability.

In patients with B_{12} -responsive methylmalonic acidemia, excretion of methylmalonic acid in the urine is significantly decreased by the administration of pharmacological doses of cyanocobalamin [3, 101]. We have employed the method of admission to the general clinical research center (GCRC) and measurement of total MMA excretion for 5 days, the first 2 control days and the next 3 reflecting daily injection of 1 mg of hydroxocobalamin or cyanocobalamin. This method has elucidated the true status in patients in whom the results of casual specimens under varying conditions in the clinic or in acute admission to hospital are confusing. A similar protocol has recently been published [17]. A decrease of mean urine or plasma MMA concentrations of 50 percent was deemed responsiveness.

The correlation of B_{12} responsiveness with prognosis is clear [30]; all but four of 25 children who responded to B_{12} were alive, while 11 of 20 who did not respond to B_{12} died. The first B_{12} -responsive patient was well at nine years when reported [102], and was 14 at the most recently reported follow up.

Those who respond are treated with B_{12} in doses sufficient to keep concentrations of methylmalonic acid minimal. B_{12} -responsive patients may do very well with modest protein restriction, growing and developing normally over the long term and tolerating childhood illnesses [103]. Most Cbl A patients can be expected to respond clinically to B_{12} , while about half of the Cbl B patients respond [30]; mut^0 and most mut^- patients have not responded despite *in vitro* evidence of responsiveness. Nevertheless, some mut^- patients will respond to B_{12} ; so they all should be tested [104].

Patients who do not respond to B_{12} are treated with a diet designed to keep the precursors of methylmalonic acid at a manageable level [23, 105]. This is complicated because isoleucine, threonine, methionine, and valine are all essential for normal growth and development. Therefore, optimal therapy consists of a diet containing the minimal

requirements of these amino acids for optimal growth and no more. The rest of the calories can be made up of a diet containing fat and carbohydrate, with or without other amino acids. The amount of protein necessary to accomplish this must be individualized. Under conditions of limited intake of protein, caloric intake must be generous. We have found that alanine supplementation is useful in this disorder and may replace a mixture of amino acids [106]. The management of such a patient is not easy. It requires enormous commitment on the part of parents, physicians, and nutritionists. Furthermore, treatment must be monitored by periodic quantitative assay concentrations of methylmalonic acid to ensure optimal control, and of plasma amino acids to ensure the avoidance of protein malnutrition. Nevertheless, it may be successful. The reward in normal development may be high. In 25 years of experience with 66 patients with methylmalonic acidemia, Saudubray and colleagues [107] pointed out that 29 of 50 B_{12} -unresponsive patients died, most of them prior to 1985, and only three after 1985. Of 21 living patients, most were judged to have had good or very good results. Treatment after 1985 reflected very rigid restriction of protein [105, 108], the addition of carnitine and the use of metronidazole [109, 110]. Not only was survival improved, but the number and severity of metabolic decompensations were decreased [108].

Propionic acid is synthesized by intestinal bacteria, and this may be an important source of propionate and methylmalonate in these patients [110]. Treatment with neomycin or metronidazole may reduce levels of propionic and methylmalonic acids in body fluids [109–111]. Doses of metronidazole have ranged from 10 to 20 mg/kg per day and have been divided into three doses. Neomycin has been used in a dose of 50 mg/kg. Other antibiotics, such as bacitracin, paromycin, clindamycin, or vancomycin, may be useful in acute situations. Lincomycin was not effective [111]. In our experience intermittent antibacterial therapy has been useful, suggesting that clonal populations of propionate-forming bacteria may be intermittently present in some patients. An effect of antibiotic treatment on metabolite accumulation may be especially useful during a crisis of metabolic decompensation. A sudden increase in methylmalonic acid excretion unaccompanied by dietary change or stimulus for catabolism may suggest a bacterial source and an argument for neomycin or metronidazole.

An increase in the excretion of metabolites of propionate during fasting suggests the mobilization of odd-chain fatty acids from lipid stores [112]. The therapeutic implication is the avoidance of fasting and the use of intravenous calories when the oral route is not available.

In the management of the acute ketoacidotic crisis, a program of aggressive fluid and electrolyte therapy is essential as set out for propionic acidemia (Chapter 2). In addition, in MMA, advantage can be taken of the very effective excretion of methylmalonate by the kidney [109], which is much more efficient than peritoneal dialysis, by aggressive intravenous hydration (150–200 mL/kg of

water containing 10 percent glucose and initially isotonic NaHCO_3 until the acidosis is corrected). Anabolism may be promoted by the use of insulin and glucose, or the acute use of growth hormone. Vomiting may be relieved with ondansetron (0.15 mg/kg over 15 minutes, intravenously, up to three times a day).

Carnitine has been a useful adjunct to chronic maintenance therapy, removing propionyl groups as carnitine ester [109, 113] and diminishing the propensity of these patients to abnormal ketogenesis [114]. We have found parenteral carnitine in doses of 300 mg/kg very useful in the acute crisis.

Human growth hormone may be useful in adjunctive therapy in this and other organic acidemias [115]. Promotion of anabolism may diminish the propensity for catabolism, and thus the acute catabolic response to infection, stress, or protein intake. In our hands, protein requirements have increased without increase in metabolite excretion. Growth has been rewarding, as well as increase in lean body mass and decrease in adipose tissue.

The fact that so many patients with mut^0 disease die in infancy and that survivors have so often had severely impaired mental development [30, 31] has led to consideration of transplantation of liver [32, 116]. Our experience [32] has indicated that liver transplantation does not halt or reverse relentless progression to renal failure. Most such patients will have had evidence of renal impairment at the time liver transplantation is considered. It makes sense to treat such a patient with combined transplantation of liver and kidney. That decision is not so clear in the case of an infant.

Our experience with liver transplantation also indicates that the procedure does not stop the progression of late onset neurologic disease [32], although it completely does away with recurrent attacks of ketoacidotic metabolic imbalance. High concentration of methylmalonic acid in the CSF does not decrease with liver transplantation (Nyhan, unpublished data) [117]. It is of interest that high CSF concentrations of methylmalonic acid have also been observed in patients with cobalamin deficiency [118]. Transplantation of the liver also did not prevent the occurrence of infarction of the basal ganglia during an episode of pneumonia unassociated with metabolic imbalance [119]. Neurologic deterioration as manifested by a cerebellar stroke was also observed in a five-year-old boy who had a combined liver kidney transplantation [120]. In a review of the literature, the authors assembled 27 patients with solid organ transplants [121]; 15 had liver and six combined liver and kidney transplantation. Tacrolimus toxicity was pointed out to be a confounding feature in the interpretation of neurologic complications, but this is treated by reducing dosage. There were five deaths, four from infection, but one died in acidosis. It is contrary to general experience that metabolic decompensation does not occur following liver transplantation.

In a novel approach to the treatment of hyperammonemia in the acute crisis of infantile methylmalonic

acidemia, Gebhardt and colleagues [121] used carbamyl-glutamate, an activator of carbamylphosphate synthetase that has been used to treat N-acetylglutamate synthetase deficiency [122]. Hyperammonemia was successfully reversed in the patient treated.

REFERENCES

1. Oberholzer VG, Levin B, Burgess EA, Young WF. Methylmalonic aciduria: an inborn error of metabolism leading to chronic metabolic acidosis. *Arch Dis Child* 1967; **42**: 492.
2. Stokke O, Eldjarn L, Norum KR *et al*. Methylmalonic acidemia: a new inborn error of metabolism which may cause fatal acidosis in the neonatal period. *Scand J Clin Lab Invest* 1967; **20**: 313.
3. Rosenberg LE, Lilljeqvist A-C, Hsia YE. Methylmalonic aciduria: an inborn error leading to metabolic acidosis, long chain ketonuria and intermittent hyperglycinemia. *N Engl J Med* 1968; **278**: 1319.
4. Rosenberg LE, Lilljeqvist A-C, Hsia YE. Methylmalonic aciduria: metabolic block localization and vitamin B_{12} dependency. *Science* 1968; **162**: 805.
5. Mudd SH, Levy HL, Abeles RH. A derangement in B_{12} metabolism leading to homocystinemia, cystathioninemia and methylmalonic aciduria. *Biochem Biophys Res Commun* 1969; **35**: 1121.
6. Higginbottom MC, Sweetman L, Nyhan WL. A syndrome of methylmalonic aciduria, homocystinuria, megaloblastic anemia and neurologic abnormalities in a vitamin B_{12} -deficient breast-fed infant of a strict vegetarian. *N Engl J Med* 1978; **299**: 317.
7. Barshop BA, Woff J, Nyhan WL *et al*. Transcobalamin II deficiency presenting with methylmalonic aciduria and homocystinuria and abnormal absorption of cobalamin. *Am J Med Genet* 1990; **35**: 222.
8. Bikker H, Bakker HD, Abeling NGGM *et al*. A homozygous nonsense mutation in the methylmalonyl-CoA epimerase gene (MCEE) results in mild methylmalonic aciduria. *Hum Mutat* 2006; **27**: 640.
9. Gradinger AB, Belair C, Worgan LC *et al*. Atypical methylmalonic acidurias; frequency of mutations in the methylmalonyl CoA epimerase gene (MCEE). *Hum Mutat* 2007; **28**: 1045.
10. Yano S, Li L, Le TP *et al*. Infantile mitochondrial DNA depletion syndrome associated with methylmalonic aciduria and 3-methylcrotonyl-CoA and propionyl-CoA carboxylase deficiencies in two unrelated patients: a new phenotype of mtDNA depletion syndrome. *J Inher Metab Dis* 2003; **26**: 481.
11. Elpeleg O, Miller C, Hershkovitz E *et al*. Deficiency of ADP-forming succinyl-CoA synthase activity is associated with encephalomyopathy and mitochondrial depletion. *Am J Hum Genet* 2005; **76**: 1081.
12. Ostergaard E, Hansen FJ, Sorensen N *et al*. Mitochondrial encephalomyopathy with elevated methylmalonic acid is caused by *SUCLA2* mutations. *Brain* 2007; **130**: 853.

13. Ostergaard E, Christensen E, Kristensen E *et al.* Deficiency of the α subunit of succinate-CoA ligase causes fatal infantile lactic acidosis with mtDNA depletion. *Am J Hum Genet* 2007; **81**: 383.
14. Morrow G, Barness LA, Cardinale GJ *et al.* Congenital methylmalonic acidemia: enzymatic evidence for two forms of the disease. *Proc Natl Acad Sci USA* 1969; **63**: 191.
15. Ledley FD, Lumetta M, Nguyen PN *et al.* Molecular cloning of L-methylmalonyl-CoA mutase: gene transfer and analysis of mut cell lines. *Proc Natl Acad Sci USA* 1988; **85**: 3518.
16. Ledley FD, Lumetta MR, Zoghbi HY *et al.* Mapping of human methylmalonyl CoA mutase (MUT) locus on chromosome 6. *Am J Hum Genet* 1988; **42**: 839.
17. Fowler B, Leonard JV, Baumgartner MR. Causes of and diagnostic approach to methylmalonic acidurias. *J Inherit Metab Dis* 2008; **31**: 350.
18. Lempp TJ, Suomalainen T, Siegenthaler R *et al.* Mutation and biochemical analysis of 19 probands with mut^0 and 13 with mut^- methylmalonic aciduria: identification of seven novel mutations. *Mol Genet Metab* 2007; **90**: 284.
19. Worgan LC, Niles K, Tirone JC *et al.* Spectrum of mutation in mut methylmalonic acidemia and identification of a common Hispanic mutation and haplotype. *Hum Mutat* 2006; **27**: 31.
20. Rosenblatt DS, Fenton WA. Inborn errors of cobalamin metabolism. In: Banerjee R (ed.). *Chemistry and Biology of B₁₂*. New York: Wiley, 1999: 367.
21. Lindblad B, Lindblad BS, Olin P *et al.* Methylmalonic acidemia: a disorder associated with acidosis, hyperglycemia and hyperlactatemia. *Acta Paediatr Scand* 1968; **57**: 417.
22. Morrow G, Barness LA, Auerbach VH *et al.* Observations on the coexistence of methylmalonic acidemia and glycemia. *J Pediatr* 1969; **74**: 680.
23. Nyhan WL, Fawcett N, Ando T *et al.* Response to dietary therapy in B₁₂ unresponsive methylmalonic acidemia. *Pediatrics* 1973; **51**: 539.
24. Cathlineau L, Briard P, Ogier H *et al.* Occurrence of hyperammonemia in the course of 17 cases of methylmalonic acidemia. *J Pediatr* 1978; **99**: 279.
25. Packman S, Mahoney MJ, Tanaka K, Hsia YE. Severe hyperammonemia in a newborn infant with methylmalonyl-CoA mutase deficiency. *J Pediatr* 1978; **92**: 769.
26. Yu A, Sweetman L, Nyhan WL. The pathogenetic mechanism of recurrent mucocutaneous candidiasis in a patient with methylmalonic acidemia (MMA). *Clin Res* 1981; **29**: 124A.
27. Choy YS, Pertiwi AKD, Zabadah Y, Noor Farizah I. Methylmalonic acidemia-associated birth defects and atypical presentations. *J Inherit Metab Dis* 2002; **25** (Suppl.): 47.
28. Shevell MA, Matiaszuk N, Ledley FD, Rosenblatt DS. Varying neurological phenotypes among mut^0 and mut^- patients with methylmalonyl CoA mutase deficiency. *Am J Med Genet* 1993; **45**: 619.
29. Hauser NS, Scott MP, Gropman AL *et al.* The functional phenotype of an inborn error of metabolism: outlining impairment and disability of methylmalonic acidemia. *Mol Genet Metab* 2010; **99**: 187 (Abstr.).
30. Matsui SM, Mahoney MJ, Rosenberg LE. The natural history of the inherited methylmalonic acidemias. *N Engl J Med* 1983; **308**: 857.
31. Nicolaides P, Leonard J, Surtees R. Neurological outcome of methylmalonic acidemia. *Arch Dis Child* 1998; **78**: 508.
32. Nyhan WL, Gargus J, Boyle K *et al.* Progressive neurologic disability in methylmalonic acidemia despite transplantation of the liver. *Eur J Pediatr* 2002; **161**: 377.
33. Thompson GN, Christodoulou J, Danks DM. Metabolic stroke in methylmalonic acidemia. *J Pediatr* 1989; **115**: 499.
34. Korf B, Wallman JK, Levy HL. Bilateral lucency of the globus pallidus complicating methylmalonic acidemia. *Ann Neurol* 1986; **20**: 364.
35. Heidenreich R, Natowicz M, Hainline BE *et al.* Acute extrapyramidal syndrome in methylmalonic acidemia: metabolic stroke involving the globus pallidus. *J Pediatr* 1988; **113**: 1022.
36. Brismar J, Ozand PT. CT and MR of the brain in disorders of the propionate and methylmalonate metabolism. *Am J Neuroradiol* 1994; **15**: 1459.
37. de Sousa C, Piesowicz AT, Brett EM, Leonard JV. Focal changes in the globus pallidus associated with neurological dysfunction in methylmalonic acidemia. *Neuropediatrics* 1989; **20**: 199.
38. Roodhooft AM, Baumgartner ER, Martin JJ *et al.* Symmetrical necrosis of the basal ganglia in methylmalonic acidemia. *Eur J Pediatr* 1990; **149**: 582.
39. Yamaguchi K, Hirabayashi K, Honma K. Methylmalonic acidemia: brain lesions in a case of vitamin B₁₂ non-responsive (mut^0) type. *Clin Neuropathol* 1995; **12**: 216.
40. Andreula CF, Deblasi R, Carella A. CT and MRI studies of methylmalonic acidemia. *Am J Neuroradiol* 1991; **12**: 410.
41. Bousounis DP. Methylmalonic aciduria resulting in globus pallidus necrosis. *Ann Neurol* 1988; **24**: 302.
42. Burlina AP, Manara R, Calderone M *et al.* Diffusion-weighted imaging in the assessment of neurological damage in patients with methylmalonic aciduria. *J Inherit Metab Dis* 2003; **26**: 417.
43. Nyhan WL, Wulfeck BB, Tallal P, Marsden DL. Metabolic correlates of learning disability. In: Paul NW (ed.). *Research in Infant Assessment*. White Plains, NY: March of Dimes Birth Defects Foundation, 1989 (*Birth Defects* **25**: 153).
44. Harting I, Seitz A, Geb S *et al.* Looking beyond the basal ganglia: the spectrum of MRI changes in methylmalonic acidemia. *J Inherit Metab Dis* 2008; **31**: 368.
45. Dave P, Curless RG, Steinman L. Cerebellar hemorrhage complicating methylmalonic and propionic acidemia. *Arch Neurol* 1984; **41**: 1293.
46. Sheldon B, Sheldon K, Sheldon P, Sheldon J. Memory of Andrew M Sheldon, MMA. *OAA Newsletter* 2003; **13**: 19.
47. Wolff JA, Strom C, Griswold W *et al.* Proximal renal tubular acidosis in methylmalonic acidemia. *J Neurogenet* 1985; **2**: 31.
48. Broyer M, Guesry P, Burgess E-A *et al.* Acidémie methylmalonique avec néphropathie hyperuricémique. *Arch Franc Pediatr* 1974; **31**: 543.
49. Rutledge SL, Geraghty M, Mroczek E *et al.* Tubulointerstitial nephritis in methylmalonic acidemia. *Pediatr Nephrol* 1993; **7**: 81.

50. Walter JH, Michalski A, Wilson WM *et al.* Chronic renal failure in methylmalonic acidemia. *Eur J Pediatr* 1989; **148**: 344.
51. Gonwa TA, Mai ML, Melton LB *et al.* End-stage renal disease (ESRD) after orthotopic liver transplantation (OLT) using calcineurin-based immunotherapy: risk of development and treatment. *Transplantation* 2001; **72**: 1934.
52. Kahler SG, Sherwood WG, Woolf D *et al.* Pancreatitis in patients with organic acidemias. *J Pediatr* 1994; **124**: 239.
53. Shevell MI, Matiaszuk N, Ledley FD, Rosenblatt DS. Varying neurological phenotypes among *mut⁰* and *mut²* patients with methylmalonyl-CoA mutase deficiency. *Am J Med Genet* 1993; **45**: 619.
54. Lind S, Westgren M, Angelin B, von Dobeln U. Successful pregnancy in a young woman with methylmalonic acidemia and a two-year follow-up of the child. *J Inher Metab Dis* 2002; **25** (Suppl.): 48.
55. Deodato F, Rizzo C, Boenzi S *et al.* Successful pregnancy in a woman with *mut2* methylmalonic acidemia. *J Inher Metab Dis* 2002; **25**: 133.
56. Ledley FD, Levy HL, Shih VE *et al.* Benign methylmalonic aciduria. *N Engl J Med* 1984; **311**: 1015.
57. Sniderman LC, Lambert M, Giguere R *et al.* Outcome of individuals with low-moderate methylmalonic aciduria detected through a neonatal screening program. *J Pediatr* 1999; **134**: 675.
58. Mayatepek E, Hoffmann GF, Baumgartner R *et al.* Atypical vitamin B₁₂-unresponsive methylmalonic aciduria in sibship with severe progressive encephalomyelopathy: a new genetic disease? *Eur J Pediatr* 1996; **155**: 398.
59. Dudley J, Allen J, Tizard J, McGraw M. Benign methylmalonic acidemia in a sibship with distal renal tubular acidosis. *Pediatr Nephrol* 1998; **12**: 564.
60. Gravel RA, Mahoney MJ, Ruddle FH, Rosenberg LE. Genetic complementation in heterokaryons of human fibroblasts defective in cobalamin metabolism. *Proc Natl Acad Sci USA* 1975; **72**: 3181.
61. Willard HF, Mellman IS, Rosenberg LE. Genetic complementation among inherited deficiencies of methylmalonyl-CoA mutase activity: evidence for a new class of human cobalamin mutant. *Am J Hum Genet* 1978; **30**: 1.
62. Mahoney MJ, Rosenberg LE, Lindblad B *et al.* Prenatal diagnosis of methylmalonic aciduria. *Acta Paediatr Scand* 1975; **64**: 44.
63. Naylor G, Sweetman L, Nyhan WL *et al.* Isotope dilution analysis of methylcitric acid in amniotic fluid for the prenatal diagnosis of propionic and methylmalonic acidemia. *Clin Chim Acta* 1980; **107**: 175.
64. Trefz FK, Schmidt H, Tauscher B *et al.* Improved prenatal diagnosis of methylmalonic acidemia: mass fragmentography of methylmalonic acid in amniotic fluid and maternal urine. *Eur J Pediatr* 1981; **137**: 261.
65. Zinn AB, Hine DG, Mahoney MJ, Tanaka K. The stable isotope dilution method for measurement of methylmalonic acid: a highly accurate approach to the prenatal diagnosis of methylmalonic acidemia. *Pediatr Res* 1982; **16**: 740.
66. Sweetman L, Naylor G, Ladner T *et al.* Prenatal diagnosis of propionic and methylmalonic acidemia by stable isotope dilution analysis of methylcitric and methylmalonic acids in amniotic fluids. In: Schmidt HL, Förstel K (eds). *Stable Isotopes*. Amsterdam: Elsevier Scientific, 1982: 287.
67. Windelberg A, Arseth O, Kvalheim G, Ueland PM. Automated assay for the determination of methylmalonic acid, total homocysteine, and related amino acids in human serum or plasma by means of methylchloroformate derivatization and gas chromatography-mass spectrometry. *Clin Chem* 2005; **51**: 11.
68. Ampola MG, Mahoney JJ, Nakamura E, Tanaka K. Prenatal therapy of a patient with vitamin B₁₂-responsive methylmalonic acidemia. *N Engl J Med* 1975; **293**: 313.
69. Stabler SP, Allen RH, Barrett RE *et al.* Cerebrospinal fluid methylmalonic acid levels in normal subjects and patients with cobalamin deficiency. *Neurology* 1991; **41**: 1627.
70. Ando T, Rasmussen K, Nyhan WL *et al.* Propionic acidemia in patients with ketotic hyperglycinemia. *J Pediatr* 1971; **78**: 827.
71. Ando T, Rasmussen K, Nyhan WL, Hull D. 3-hydroxypropionate: significance of oxidation of propionate in patients with propionic acidemia and methylmalonic acidemia. *Proc Natl Acad Sci USA* 1972; **69**: 2807.
72. Ando T, Rasmussen K, Wright JM, Nyhan WL. Isolation and identification of methylcitrate, a major metabolic product of propionate in patients with propionic acidemia. *J Biol Chem* 1972; **247**: 2200.
73. Pitt JJ, Eggington M, Kahler SG. Comprehensive screening of urine samples for inborn errors of metabolism by electrospray tandem mass spectrometry. *Clin Chem* 2002; **48**: 1970.
74. Magera MJ, Helgeson JK, Matern D, Rinaldo P. Methylmalonic acid measured in plasma and urine by stable-isotope dilution and electrospray tandem mass spectrometry. *Clin Chem* 2000; **46**: 1804.
75. Bolann BJ, Solli JD, Schneede J *et al.* Evaluation of indicators of cobalamin deficiency defined as cobalamin-induced reduction in increased serum methylmalonic acid. *Clin Chem* 2000; **46**: 1744.
76. Wolff JA, Sweetman L, Nyhan WL. The role of lipid in the management of methylmalonic acidemia: administration of linoleic acid does not increase excretion of methylmalonic acid. *J Inher Metab Dis* 1985; **8**: 100.
77. Morrow G, Mahoney MJ, Mathews C, Lebowitz J. Studies of methylmalonyl coenzyme A carboxymutase activity in methylmalonic acidemia. I. Correlation of clinical, hepatic and fibroblast data. *Pediatr Res* 1975; **9**: 641.
78. Willard HF, Rosenberg LE. Inherited deficiency of human methylmalonyl CoA mutase activity: reduced affinity of mutant apoenzyme for adenosylcobalamin. *Biochem Biophys Res Commun* 1977; **78**: 927.
79. Willard HF, Rosenberg LE. Inherited methylmalonyl CoA mutase apoenzyme deficiency in human fibroblasts: evidence for allelic heterogeneity, genetic compounds, and co-dominant expression. *J Clin Invest* 1980; **65**: 690.
80. Kolhouse JF, Utley C, Fenton WA, Rosenberg LE. Immunochemical studies on cultured fibroblasts from patients with inherited methylmalonic acidemia. *Proc Natl Acad Sci USA* 1981; **78**: 7737.
81. Fenton WA, Hack AM, Kraus JP, Rosenberg LE. Immunochemical studies of fibroblasts from patients with methylmalonyl-

- CoA mutase apoenzyme deficiency: detection of a mutation interfering with mitochondrial import. *Proc Natl Acad Sci USA* 1987; **84**: 1421.
82. Mahoney MJ, Rosenberg LE, Mudd SH, Uhlendorf BW. Defective metabolism of vitamin B₁₂ in fibroblasts from patients with methylmalonic aciduria. *Biochem Biophys Res Commun* 1971; **44**: 375.
 83. Rosenberg LE, Lilljeqvist AC, Hsia YE, Rosenbloom FM. Vitamin B₁₂-dependent methylmalonic aciduria: defective metabolism in cultured fibroblasts. *Biochem Biophys Res Commun* 1969; **37**: 607.
 84. Willard HF, Ambani LM, Hart AC *et al*. Rapid prenatal and postnatal detection of inborn errors of propionate, methylmalonate, and cobalamin metabolism a sensitive assay using cultured cells. *Hum Genet* 1976; **34**: 277.
 85. Morrow G, Revsin B, Mathews C, Giles H. A simple rapid method for prenatal detection of defects in propionate metabolism. *Clin Genet* 1976; **10**: 218.
 86. Morrow G, Barness LA, Cardinale GJ. Congenital methylmalonic acidemia: enzymatic evidence for two forms of disease. *Proc Natl Acad Sci USA* 1975; **72**: 2799.
 87. Mahoney MJ, Hart AC, Steen VD, Rosenberg LE. Methylmalonic acidemia: biochemical heterogeneity in defects of 5'-deoxyadenosylcobalamin synthesis. *Proc Natl Acad Sci USA* 1975; **72**: 2799.
 88. Fenton WA, Rosenberg LE. Genetic and biochemical analysis of human cobalamin mutants in cell culture. *Ann Rev Genet* 1978; **12**: 223.
 89. Fenton WA, Rosenberg LE. The defect in the cbl B class of human methylmalonic acidemia: deficiency of cobalamin adenosyltransferase activity in extracts of cultured fibroblasts. *Biochem Biophys Res Commun* 1981; **98**: 283.
 90. Jansen R, Kalousek F, Fenton WA *et al*. Cloning of full-length methylmalonyl-CoA mutase: gene transfer and analysis of mut cell lines. *Proc Natl Acad Sci USA* 1988; **85**: 3618.
 91. Crane AM, Ledley FD. Clustering of mutations in methylmalonyl CoA mutase associated with mut² methylmalonic acidemia. *Am J Hum Genet* 1994; **55**: 42.
 92. Crane AM, Jansen R, Andrews E, Ledley FD. Cloning and expression of a mutant methylmalonyl coenzyme A mutase with altered cobalamin affinity that causes mut⁻ methylmalonic aciduria. *J Clin Invest* 1992; **89**: 385.
 93. Jansen R, Ledley FD. Heterozygous mutations at the mut locus in fibroblasts with mut⁰ methylmalonic acidemia identified by PCR cDNA cloning. *Am J Hum Genet* 1990; **47**: 808.
 94. Ledley FD, Crane AM, Lumetta M. Heterogeneous alleles and expression of methylmalonyl CoA mutase in mut methylmalonic acidemia. *Am J Hum Genet* 1990; **47**: 808.
 95. Wilkemeyer MG, Crane AM, Ledley FD. Differential diagnosis of mut and cbl methylmalonic aciduria by DNA-mediated gene transfer in primary fibroblasts. *J Clin Invest* 1991; **87**: 915.
 96. Fenton WA, Hack AM, Kraus JP, Rosenberg LE. Immunochemical studies of fibroblasts from patients with methylmalonyl-CoA mutase apoenzyme deficiency: detection of a mutation interfering with mitochondrial import. *Proc Natl Acad Sci USA* 1987; **84**: 1421.
 97. Martinez MA, Rincon A, Desviat LR *et al*. Genetic analysis of three genes causing isolated methylmalonic acidemia; identification of 21 novel allelic variants. *Mol Genet Metab* 2005; **84**: 317.
 98. Lerner-Ellis JP, Gradinger AB, Watkins D *et al*. Mutation and biochemical analysis of patients belonging to the cblB complementation class of vitamin B₁₂ dependent methylmalonic aciduria. *Mol Genet Metab* 2006; **87**: 219.
 99. Chandler RJ, Zervas PM, Shanske S *et al*. Mitochondrial dysfunction in mut methylmalonic acidemia. *FASEB J* 2009; **23**: 1252.
 100. Valayannopoulos V, Hubert L, Benoist JF *et al*. Multiple OXPHOS deficiency in the liver of a patient with CblA methylmalonic aciduria sensitive to vitamin B₁₂. *J Inherit Metab Dis* 2009; **32**: 159.
 101. Lindblad B, Lindstrand K, Svenberg B, Zetterstrom R. The effect of cobamide coenzyme in methylmalonic acidemia. *Acta Paediatr Scand* 1969; **58**: 178.
 102. Hsia YE, Scully K, Lilljeqvist AC, Rosenberg LE. Vitamin B₁₂-dependent methylmalonic aciduria. *Pediatrics* 1970; **46**: 497.
 103. Morrow III G, Burkel GM. Long-term management of a patient with vitamin B₁₂-responsive methylmalonic acidemia. *J Pediatr* 1980; **96**: 425.
 104. Hörster F, Baumgartner MR, Viardot C *et al*. Long-term outcome in methylmalonic aciduria is influenced by the underlying defect (mut⁰, mut⁻, cblA, cblB). *Pediatr Res* 2007; **62**: 225.
 105. Ney DN, Bay C, Saudubray J-M *et al*. An evaluation of protein requirements in methylmalonic acidemia. *J Inherit Metab Dis* 1985; **8**: 132.
 106. Kelts DG, Ney D, Bay C *et al*. Studies on requirements for amino acids in infants with disorders of amino acid metabolism. I. Effects of alanine. *Pediatr Res* 1985; **19**: 86.
 107. Van der Meer SB, Poggi F, Spada M *et al*. Clinical outcome of long-term management of patients with vitamin B₁₂-unresponsive methylmalonic acidemia. *J Pediatr* 1994; **125**: 903.
 108. Thompson GN, Chalmers RA, Walter JH *et al*. The use of metronidazole in the management of methylmalonic and propionic acidemias. *Eur J Pediatr* 1990; **149**: 792.
 109. Walter JH, Thompson GN, Leonard JV *et al*. Contribution of amino acid catabolism to propionate production in methylmalonic acidemia. *Lancet* 1989; **1**: 1298.
 110. Snyderman S, Sansaricq C, Norton P *et al*. The use of neomycin in the treatment of methylmalonic acidemia. *Pediatrics* 1972; **50**: 925.
 111. Thompson GN, Chalmers RA. Increased urinary metabolite excretion during fasting in disorders of propionate metabolism. *Pediatr Res* 1990; **27**: 413.
 112. Saudubray JM, Ogier H, Charpentier C *et al*. Neonatal management of organic acidurias. Clinical update. *J Inherit Metab Dis* 1984; **7**: 1.
 113. Chalmers RA, Roe CR, Stacey TE, Hoppel CL. Urinary excretion of L-carnitine and acylcarnitines by patients with disorders of organic acid metabolism: evidence for secondary insufficiency of L-carnitine. *Pediatr Res* 1984; **18**: 1325.
 114. Wolff JA, Carroll JE, Thuy LP *et al*. Carnitine reduces fasting ketogenesis in patients with disorders of propionate metabolism. *Lancet* 1986; **1**: 289.

115. Marsden D, Barshop BA, Capistrano-Estrada S *et al*. Anabolic effect of human growth hormone: management of inherited disorders of catabolic pathways. *Biochem Med Metab Biol* 1994; **52**: 145.
116. Van't Hoff WG, McKiernan PJ, Surtees RAH, Leonard JV. Liver transplantation for methylmalonic acidemia. *Eur J Pediatr* 1999; **158**: S70.
117. Kaplan P, Ficicioglu C, Mazur A *et al*. Liver transplantation is not curative for methylmalonic acidopathy caused by methylmalonyl-CoA mutase deficiency. *Mol Genet Metab* 2006; **88**: 322.
118. Van Asselt DZ, Karlietis MH, Poels PJ *et al*. Cerebrospinal fluid methylmalonic acid concentrations in neurological patients with low and normal serum cobalamin concentrations. *Acta Neurol Scand* 1998; **97**: 413.
119. Chakrapani A, Sivakumar P, McKiernan PJ, Leonard JV. Metabolic stroke in methylmalonic acidemia five years after liver transplantation. *J Pediatr* 2002; **140**: 261.
120. McGuire PJ, Lim-Melia E, Diaz GA *et al*. Combined liver-kidney transplant for the management of methylmalonic aciduria: a case report and review of the literature. *Mol Genet Metab* 2008; **93**: 22.
121. Gebhardt B, Vlaho S, Fischer D *et al*. N-carbamylglutamate enhances ammonia detoxification in a patient with decompensated methylmalonic aciduria. *Mol Genet Metab* 2003; **79**: 303.
122. Bachman C, Colombo JP, Jaggi K. N-acetylglutamate synthetase (NAGS) deficiency: diagnosis, clinical observation and treatment. *Adv Exp Med Biol* 1982; **153**: 313.

Methylmalonic aciduria and homocystinuria (cobalamin C and D disease)

Introduction	33	Treatment	37
Clinical abnormalities	34	References	38
Genetics and pathogenesis	36		

MAJOR PHENOTYPIC EXPRESSION

Megaloblastic anemia; failure to thrive; developmental delay; excretion of homocystine and methylmalonic acid; and defective activities of both methylmalonyl CoA mutase and methionine synthase.

INTRODUCTION

Patients with methylmalonic aciduria and homocystinuria have defective metabolism of cobalamin to both cofactors, methylcobalamin and deoxyadenosylcobalamin [1–4]. Accordingly, the activities of methionine synthase and methylmalonyl CoA mutase are defective (see [Figure 4.1](#)). Patients with impaired synthesis of methylcobalamin and deoxyadenosylcobalamin fall into two distinct complementation groups designated Cbl C and Cbl D. Another group of patients designated Cbl F have defective transport of free cobalamin out of lysosomes. The differential diag-

nosis of methylmalonic acidemia and homocystinuria is given in [Table 4.1](#).

The Cbl C disease is the most common, and its clinical picture is heterogeneous, but a majority of patients have early onset disorder with major dysfunction and a median age at death of two months [5].

The disease gene (*MMACHC*) has been cloned [6] and over 40 mutations have been identified [5–7]. The most common mutation c.271dupA accounted for 55 percent of southern European and French Canadian patients [5, 8, 9]. This and two other mutations, c.394C>T and c.331C>T accounted for most of the populations studied; indicating

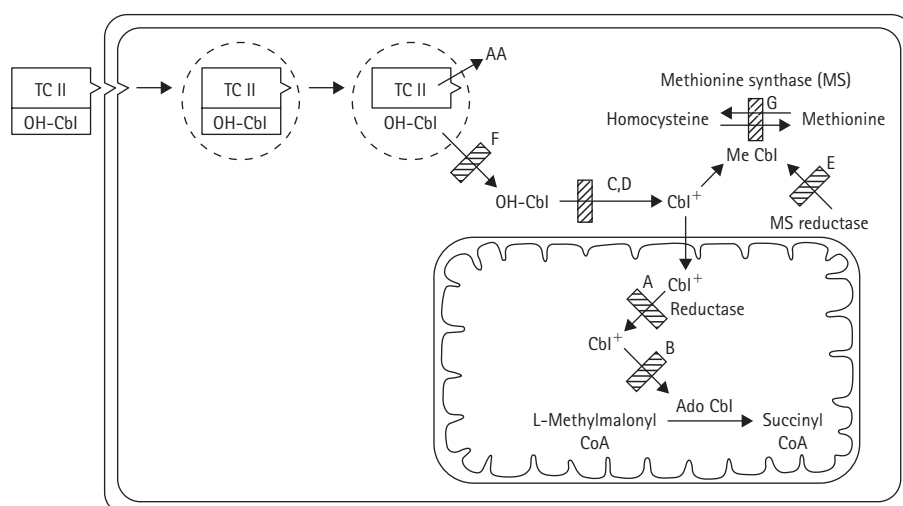


Figure 4.1 Cobalamin transport and metabolism sites of the defects in complementation groups A to G.

Table 4.1 Differential diagnosis: methylmalonic acidemia and homocystinuria

Disorder	Methylmalonic aciduria	Homocystinuria	Methionine increase	Serum B ₁₂ low
Cobalamin (Cbl) C	+	+	0	0
Cobalamin (Cbl) D	+	+	0	0
MMA mutase apoenzyme (Mut ⁰ , Mut ⁻)	+	0	0	0
Cystathionine synthase	0	+	+	0
Methylene tetrahydrofolate reductase	0	+	0(↓)	0
Cobalamin (Cbl) E, G	0	+	0	0
Cobalamin (Cbl) F	+	0	0	0
B ₁₂ deficiency	+	+	0	+
Gastrointestinal surgery	+	±	0	+
Autoimmune-multiple endocrine deficiency antibody to parietal cells	+	±	0	+
TC II deficiency	+	±	0	+
Cobalamin enterocyte malabsorption-Immerslund-Grassbeck	+	±	0	+



Figure 4.2 JA: A four-year-old boy with Cbl C disease. He looked quite good. His magnetic resonance images are shown in Figures 4.3 and 4.4. (Images were kindly provided by Dr GM Enns of Stanford University).

that rapid search for these three mutations in infants detected by newborn screening could yield presymptomatic diagnosis and the more rapid introduction of treatment.

CLINICAL ABNORMALITIES

The clinical manifestations of Cbl C disease, often begin with megaloblastic anemia and failure to thrive (Figure 4.2) [1, 2]. Death may occur within the first six months of life [1, 3], and there may be overwhelming illness starting in the first days of life. Some patients have seizures; some have

microcephaly. Lethargy and/or irritability are prominent. Patients may be difficult to feed. Patients with onset later than the early months of life have had predominantly neurologic presentations. Anorexia, irritability, or fatigue may be seen, as well as myelopathy or dementia. Hematologic examination is like that of pernicious anemia with hypersegmented polymorphonuclear leukocytes, and sometimes thrombocytopenia, as well as the megaloblastic anemia. One patient [10] had severely impaired mental development and megaloblastic anemia; he died at seven years of age. As the numbers of patients recognized with cobalamin C disease has increased, clinical heterogeneity has become apparent [4, 11]. Retinal degeneration has been reported [12], as well as pigmentary retinopathy, which may aid in the clinical diagnosis. Another infant presented at eight months of age with hypotonia, failure to thrive, and macrocytic anemia [13]. He did not appear to see or hear, and visual and auditory evoked potentials were abnormal. Infants with neonatal onset who survive the initial episode, may have metabolic decompensation during intercurrent illness, as in other organic acidemias. A 30-year-old patient was reported [14] who presented first at 12 years of age with fatigue, ataxia, and mild incontinence, indicating involvement of the spinal cord. Seven years later, she developed peripheral nerve disease. A relapsing-remitting course suggested multiple sclerosis. At 24 years, she had deep vein thrombosis and lost the ability to walk. Six months later, she required intensive care. A 34-year-old sister with the same metabolic defect was well. Neither had hematologic abnormalities. A small group of neonates with methylmalonic acidemia and homocystinuria of the Cbl C group have presented with microangiopathy, anemia, and a hemolytic-uremic syndrome. All have died early in life [15].

Cutaneous manifestations consistent with a diagnosis of

acrodermatitis enteropathica were reported in two infants with Cbl C diseases [16]. Lesions were erythematous, superficially erosive, desquamative, and hyperkeratotic. There was associated cheilosis and perioral erosions. Lesions of this type have been attributed to nutritional deficiency in many inborn errors under treatment, but these patients presented with skin lesions at 9 days and 19 days, before nutritionally restrictive therapy had begun. On the other hand, both had very low levels of methionine in plasma: 10 $\mu\text{mol/L}$ and 1 $\mu\text{mol/L}$; so this could still represent deficiency of an essential amino acid.

Among those with Cbl C disease surviving early infancy, neurological manifestations have been prominent. Impaired mental development has been the rule [4]. Microcephaly, nystagmus, visual impairment, and retinopathy have been prominent features. Progressive neurodegenerative disease was reported [17], despite early treatment with hydroxocobalamin and improvement in the concentrations of metabolites. She had presented at 9 days of life and was treated with hydroxocobalamin within the next 2 weeks. She developed choreoathetosis and brisk reflexes at 13 months and seizures at 15 months. Acute stroke with coma has also been observed in this disease [18], although not with the frequency seen in cystathionine synthase deficiency.

In a series of 41 Portuguese and Italian patients [5], 36 had early onset disease presenting in the first year of life, and five had later onset disease. Prenatal onset abnormalities, such as microcephaly and intrauterine growth impairment, were seen in a number of patients. All of the early onset patients had failure to thrive, feeding difficulties, and episodes of acidosis, thrombocytopenia, leukopenia, or anemia. Three patients in this series had a hemolytic-anemic syndrome. With time, nearly all had cognitive/developmental delay.

Late onset more attenuated patients had mild to moderate cognitive impairment, seizures, and corticospinal tract signs. Some late onset patients have presented with neuropsychiatric symptoms. Association with vasculopathy and mitochondrial electron transport chain dysfunction has also been observed. Late onset patients in general have had better survival and responded to treatment and fewer neurological sequelae [19] than early onset patients. Two siblings had late onset hemolytic-uremic thrombotic microangiopathy [20]. They were effectively treated with hydroxocobalamin.

Neuroimaging [21, 22] revealed evidence on magnetic resonance image (MRI) of diffuse edema and dysmyelination of white matter at presentation, and volume loss of white matter with time, and communicating hydrocephalus (Figures 4.3 and 4.4). Electroencephalogram (EEG) may show epileptiform abnormalities [22]. Evoked responses display increased latency and prolonged conduction [13, 22]. In the patient with the neurodegenerative picture, there was prominent involvement of the globus pallidus on MRI and clear evidence of progression. Neurologic progression and

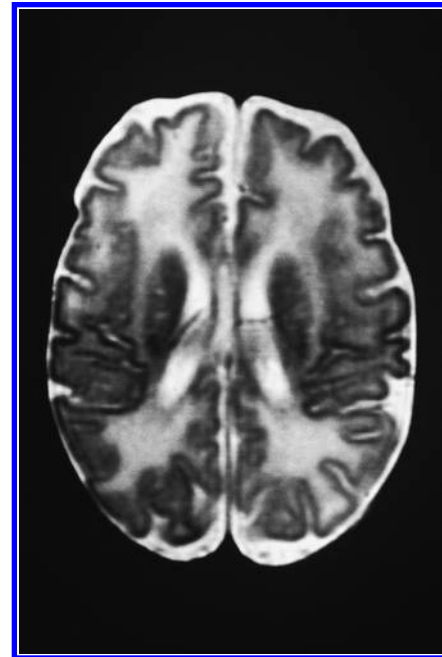


Figure 4.3 Magnetic resonance image of the head of a 5-week-old infant with Cbl C disease. Abnormal signal was consistent with white matter disease. (Images were kindly supplied by Dr GM Enns of Stanford University.)

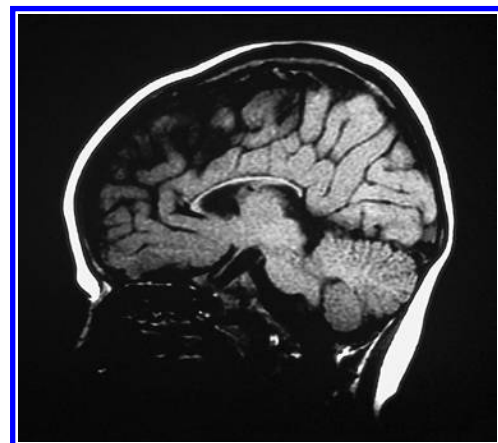


Figure 4.4 Magnetic resonance image of the brain of the same patient at 23 months. By this time, there was extensive paucity of myelination and diffuse atrophy. The corpus callosum was very thin. Neurologic progression occurred despite therapy with hydroxocobalamin. (Images were kindly provided by Dr GM Enns of Stanford University.)

cerebral atrophy were observed despite therapy with hydroxocobalamin. The neonatal MRI was normal; that of one year revealed white matter loss and ventricular enlargement and normal basal ganglia. At 15 months, both globi were hyperintense. Lesions in the basal ganglia have been observed in methylmalonic acidemia of the mut^0 and mut^- types, and in Cbl A and Cbl B disease, and in propionic acidemia. We have also observed similar

involvement of the globi pallidi in transcobalamin II deficiency. Multiple small infarcts of the basal ganglia were found in the basal ganglia of a patient with Cbl C disease who died at 22 months [15].

Only two patients have been reported with Cbl D disease; they were brothers, neither of whom was anemic [2]. The older one had impaired mental development and psychotic, and had abnormalities of cerebellar and spinal cord function, including ataxia. His two-year-old affected brother appeared well at report at two years of age. Thromboembolic complications may be observed, as in cystathionine synthase deficiency (Chapter 18).

Five patients have been reported with Cbl F disease [23, 24]. Deep tendon reflexes were accentuated and there was an intention tremor. The first two presented within the first 2 weeks of life with stomatitis, failure to thrive, and hypotonia [23]. Seizures [23, 25] were observed, as was developmental delay. There were no hematologic abnormalities in the first patient, but macrocytosis, hypersegmented polymorphonuclear leukocytes, and even pancytopenia have been observed. One infant died suddenly despite a good biochemical response to cobalamin.

GENETICS AND PATHOGENESIS

Each of the Cbl group diseases is transmitted in an autosomal recessive fashion. In each, the activity of methionine synthase is deficient, and so is that of methylmalonyl-CoA mutase (see Figure 3.1). Methionine synthase activity has been demonstrated to be restored by the addition of methylcobalamin [26, 27]. The fundamental defect in Cbl C and D disease involves a step in cobalamin processing so early that the formations of both methylcobalamin and deoxyadenosyl cobalamin are altered. In Cbl F disease, the defect has been identified in the transport step in which cobalamin in lysosomes, once TCII is split off, is normally transported out of the lysosome to begin cofactor synthesis [25]. The transporter defect is analogous to those of sialic acid storage disease and of cystine storage disease (cystinosis) (Chapter 71).

Methionine synthase activity is deficient in Cbl E and G diseases. This enzyme catalyzes the transfer of a methyl

of 5-methyltetrahydrofolate to homocysteine. Mutations in the gene coding for this enzyme cause Cbl G disease. In Cbl E disease, the mutations are in the gene for methionine synthase reductase which maintains the synthase in its reduced state.

In the presence of defective cofactor synthesis, methylmalonate and homocystine accumulate. The amounts are distinctly less than in methylmalonyl CoA mutase deficiency or cystathionine synthase deficiency (Table 4.2). The diagnosis is usually made by organic analysis of the urine, which detects methylmalonate, the most abundant metabolite. Methylcitrate and 3-hydroxypropionate are also identified in this way. Screening tests for methylmalonate (Chapter 3) are also positive, and the diagnosis may first be suspected in this way. Quantitative assay of the urinary amino acids reveals elevated amounts of homocystine. It is important for this purpose to employ fresh urine.

The amounts of homocystine are not large, and this compound is unstable in urine at room temperature. Also, proteinuria may lead to binding of homocystine, which would then be precipitated out and removed from the analysis when the urine is acidified. Screening the urine with the cyanide nitroprusside test is also positive (Chapter 18). Some patients have had hypomethioninemia and cystathioninuria [16, 28]. Homocystine may be found in the plasma by assay for total homocystine. Regardless of the method, homocystine is not found in some patients with Cbl C, and even with Cbl F disease [23].

Once the biochemical diagnosis is made, complementation analysis is performed with cultured fibroblasts incubated with ¹⁴C-propionate to determine the specific Cbl complementation group [29]. Studies of the uptake of ⁵⁷Co-cyanocobalamin by fibroblasts have indicated deficiency in the process of conversion to hydroxycobalamin and the conversion of either to methylcobalamin and deoxyadenoxylcobalamin in patients with Cbl C and D disease [26, 29]. Uptake was normal in other forms of homocystinuria and methylmalonic acidemia. Cells of patients with Cbl C utilize CN-Cbl poorly and cannot convert CN-Cbl to OH-Cbl [27, 28, 30]. This could indicate a defect at cobalamin (III) reductase, catalyzing the reduction of trivalent cobalt prior to alkylation. Concentrations of B₁₂ in the serum may be elevated [13].

The biochemical picture of methylmalonic acidemia and homocystinuria and the acute hematological and clinical neurologic picture of Cbl C disease have been encountered in the exclusively breastfed infants of strict vegan mothers [31, 32], as well as in the breastfed infants of mothers with subclinical pernicious anemia and in TCII deficiency [33]. It is also seen in the Immerslund-Grasbeck defect in ileal absorption of the B₁₂-intrinsic factor complex (Figures 4.5 and 4.6) [34]. Problems in the differential diagnosis of patients with methylmalonic acidemia were highlighted by a patient who died of mutase deficiency, ultimately diagnosed when a sibling was found to have the disease, but not before the mother was incarcerated

Table 4.2 Pathological biochemistry of the urine in Cbl C and D diseases

Metabolite	Pathological (Cbl C and D)	Normal (mmol/mol creatinine)
Urinary methylmalonate	50–700	0–2
Urinary 3-hydroxypropionate	6–30	0–24
Urinary methylcitrate	30–6	0–5
Urinary homocystine	0.08–80	0–0.01



Figure 4.5 A 12-year-old girl with the Immerslund-Grasbeck B_{12} intestinal absorptive defect [34]. She was bed-ridden, semicomatose, demented, and required intragastric feeding. She had anemia for years, but developed paraparesis at ten years. The urine had increased levels of homocystine and methylmalonate. Schilling test was abnormal with and without intrinsic factor. Treatment with hydroxocobalamin led to a remarkable improvement.



Figure 4.6 The same patient was able to walk with crutches or a walker after four months of treatment. On the left, her three-year-old sister was found to have severe anemia and was found to have the same disease. Treatment with hydroxocobalamin cured the anemia and prevented neurologic disease [34].

for homicide because a commercial clinical laboratory misidentified the propionic acid in the blood as ethylene glycol [35]. This experience points up the importance of quantification and identification by gas chromatography-mass spectrometry (GCMS) as opposed to identification based on elution times in gas chromatography.

The molecular nature of the disease has been explored since the cloning of the gene [6]. The most commonly encountered mutation c.271dupA has, when homozygous, been uniformly found to lead to the early onset severe phenotype. The c.394C>T mutation has never been encountered in homozygosity among patients with this early onset phenotype. This mutation was found in 16 percent of Italian and Portuguese alleles [5] and 24 percent of French-Canadian alleles [8, 9]. On the other hand, when associated with the c.271dupA mutation, the phenotype was usually early onset. A few late onset patients were compound heterozygous for c.271dupA gene and a missense mutation [9]. Late onset patients with hemolytic-uremic disease in the absence of neurologic disease were heterozygous for c.271dupA and a c.82-9 12del ITTTC, an intronic mutation not otherwise encountered in the database [9]. The possibility that mutation in the methylenetetrahydrofolate reductase gene (*MTHFR*) might act as a genetic modifier in Cbl C patients was explored and found not to affect age of onset, clinical phenotype, or outcome [5].

TREATMENT

Treatment of all Cbl C disease has largely been unsatisfactory. Most patients have died or been severely handicapped. The documented poor uptake of labeled cyano B_{12} by fibroblasts [26] indicated that these patients should be treated with hydroxocobalamin. Large doses, 1–1.5 mg intramuscularly (i.m.) each day, have been employed [36, 37]. Significant decreases in urinary methylmalonate have been observed and similar effects were observed in plasma homocysteine. Growth rates have become normal. Reversion to normal of abnormal visual and auditory evoked potentials have been reported [13]. Treatment with hydroxocobalamin has also been reported in Cbl E disease to resolve homocystinuria, methylmalonic aciduria, hypomethioninemia, megaloblastic anemia, and failure to thrive [38].

Supplemental uses of betaine, carnitine, and folate have been recommended. The fact that creatine synthesis from guanidinoacetate requires methyl groups provided by the conversion of methionine to homocysteine led to the finding that concentrations of guanidinoacetate are high and those of creatine low in five patients with Cbl C disease [39]. This raises the possibility that treatment with creatine may be helpful.

Patients detected by newborn screening have been diagnosed definitively by complementation analysis of cultured fibroblasts, a process that might take as long as two months. Follow up by testing for the two most common

genes, or by adding the third c.331C>T should dramatically accelerate this process and lead to early treatment. Testing for c.271dupA has been reported to accomplish early diagnosis in two patients [5].

REFERENCES

- Mudd SH, Levy HL, Abeles RH. A derangement in B₁₂ dependency leading to homocystinemia, cystathioninemia and methylmalonic aciduria. *Biochem Biophys Res Commun* 1969; **35**: 121.
- Goodman SI, Moe PG, Hammond KB *et al.* Homocystinuria with methylmalonic aciduria: two cases in sibship. *Biochem Med* 1970; **4**: 500.
- Deodata F, Boenzi S, Santorelli FM *et al.* Methylmalonic and propionic aciduria. *Am J Med Genet C* 2006; **142**: 104.
- Rosenblatt DS, Aspler AL, Shevell MI *et al.* Clinical heterogeneity and prognosis in combined methylmalonic aciduria and homocystinuria. *J Inher Metab Dis* 1997; **20**: 528.
- Noguera C, Aiello C, Cerone R *et al.* Spectrum of MMACHC mutations in Italian and Portuguese patients with combined methylmalonic Aciduria and homocystinuria cblC type. *Mol Genet Metab* 2008; **93**: 475.
- Lerner-Ellis JP, Tirone JC, Pawelek PD *et al.* Identification of the gene responsible for methylmalonic aciduria and homocystinuria, cblC type. *Nat Genet* 2006; **38**: 93.
- Yuen YP, Lai CK, Chan YW *et al.* DNA-based diagnosis of methylmalonic aciduria and homocystinuria, cblC type in a Chinese patient presenting with mild developmental delay. *Clin Chim Acta* 2007; **375**: 171.
- Marinho C, Alho I, Arduino D *et al.* GST M1/T1 and MTHFR polymorphisms as risk factors for hypertension. *Biochem Biophys Res Commun* 2007; **353**: 344.
- Morel CF, Lerner-Ellis JP, Rosenblatt DS *et al.* Combined methylmalonic aciduria and homocystinuria (cblC): phenotype-genotype correlations and ethnic-specific observations. *Mol Genet Metab* 2006; **88**: 315.
- Dillon MJ, England JM, Gompertz D *et al.* Mental retardation, megaloblastic anemia, methylmalonic aciduria and abnormal homocysteine metabolism due to an error in vitamin B₁₂ metabolism. *Clin Sci Molec Med* 1974; **47**: 43.
- Mitchell GA, Watkins D, Melancon SB *et al.* Clinical heterogeneity in cobalamin C variant of combined homocystinuria and methylmalonic aciduria. *J Pediatr* 1986; **108**: 410.
- Robb RM, Dowton SB, Fulton AB, Levy HS. Retinal degeneration in vitamin B₁₂ disorder associated with methylmalonic aciduria and sulfur amino acid abnormalities. *Am J Ophthalmol* 1984; **97**: 691.
- Mamlok RJ, Isenberg JN, Rassin DK. A cobalamin metabolic defect with homocystinuria, methylmalonic aciduria and macrocytic anemia. *Neuropediatrics* 1986; **17**: 94.
- Baumgartner EF, Fowler B, Gold R. Hereditary defect of cobalamin metabolism (cbl C) of juvenile onset with neurological features resembling multiple sclerosis. *Proceedings of the International Society of Inborn Errors of Metabolism*, Milan, 1994.
- Geraghty MT, Perlman EJ, Martin LS *et al.* Cobalamin C defect associated with hemolytic-uremic syndrome. *J Pediatr* 1992; **120**: 934.
- Howard R, Frieden IJ, Crawford D *et al.* Methylmalonic acidemia, cobalamin C type, presenting with cutaneous manifestations. *Arch Dermatol* 1997; **133**: 1563.
- Enns GM, Barkovich AJ, Rosenblatt DS *et al.* Progressive neurological deterioration and MRI changes in Cbl C methylmalonic acidemia – treated with hydroxocobalamin. *J Inher Metab Dis* 1999; **22**: 599.
- Polanco Y, Polanco FMMA. Cbl C at age 6½. *OAA Newsletter* 2002; **12**: 2.
- Ben-Omran TI, Wong H, Blaser S *et al.* Late-onset cobalamin-C disorder: a challenging diagnosis. *Am J Med Genet A* 2007; **143A**: 979.
- Van Hove JL, Van Damme-Lombaerts R, Grunewald S *et al.* Cobalamin disorder Cbl-C presenting with late-onset thrombotic microangiopathy. *Am J Med Genet* 2002; **111**: 195.
- Rossi A, Cerone R, Biancheri R *et al.* Early-onset combined methylmalonic aciduria and homocystinuria: neuroradiologic findings. *Am J Neuroradiol* 2001; **22**: 554.
- Biancheri R, Cerone R, Schiffino MC *et al.* Cobalamin (cbl) C/D deficiency: clinical neurophysiological and neuroradiologic findings in 14 cases. *Neuropediatrics* 2001; **32**: 14.
- Rosenblatt DS, Laframboise R, Pichette J *et al.* New disorder of vitamin B₁₂ metabolism (cobalamin F) presenting as methylmalonic aciduria. *Pediatrics* 1985; **78**: 51.
- Shih VE, Axel SM, Tewksbury JC *et al.* Defective lysosomal release of vitamin B₁₂ (cblF): a hereditary metabolic disorder associated with sudden death. *Am J Med Genet* 1989; **33**: 555.
- Watkins GA, Rosenblatt PS. Failure of lysosomal release of vitamin B₁₂: a new complementation group causing methylmalonic aciduria (cbl F). *Am J Hum Genet* 1986; **39**: 404.
- Mahoney MJ, Rosenberg LE, Mudd SH, Uhlenhuth BW. Defective metabolism of vitamin B₁₂ in fibroblasts from patients with methylmalonic aciduria. *Biochem Biophys Res Commun* 1971; **44**: 375.
- Mudd SH, Uhlenhuth BW, Hinds KR, Levy HL. Deranged B₁₂ metabolism: studies of fibroblasts grown in tissue culture. *Biochem Med* 1970; **4**: 215.
- Levy HL, Mudd SH, Shulman JD *et al.* A derangement in B₁₂ metabolism associated with homocystinemia, cystathioninemia, hypomethioninemia and methylmalonic aciduria. *Am J Med* 1970; **48**: 390.
- Willard HF, Mellman IS, Rosenberg LE. Genetic complementation among inherited deficiencies of methylmalonyl-CoA mutase activity: evidence for a new class of human cobalamin mutant. *Am J Hum Genet* 1978; **30**: 1.
- Mellman I, Willard HF, Youngdahl-Turner P, Rosenberg LE. Cobalamin coenzyme synthesis in normal and mutant human fibroblasts: evidence for a processing enzyme activity deficient in cbl C cells. *J Biol Chem* 1979; **254**: 11847.
- Higginbottom MC, Sweetman L, Nyhan WL. A syndrome of methylmalonic aciduria, homocystinuria, megaloblastic anemia and neurologic abnormalities in a vitamin B₁₂-deficient

- breast-fed infant of a strict vegetarian. *N Engl J Med* 1978; **299**: 317.
32. Kuhne T, Bubl R, Baumgartner R. Maternal vegan diet causing infantile neurological disorder due to vitamin B₁₂ deficiency. *Eur J Pediatr* 1991; **150**: 205.
33. Barshop BA, Wolff J, Nyhan WL *et al*. Transcobalamin II deficiency presenting with methylmalonic aciduria and homocystinuria and abnormal absorption of cobalamin. *Am J Med Genet* 1990; **35**: 222.
34. Al Essa M, Sakati NA, Dabbagh O *et al*. Inborn error of vitamin B₁₂ metabolism: a treatable cause of childhood dementia/paralysis. *J Child Neurol* 1998; **13**: 239.
35. Shoemaker JD, Lynch RE, Hoffmann JW, Sly WS. Misidentification of propionic acid as ethylene glycol in a patient with methylmalonic acidemia. *J Pediatr* 1992; **120**: 417.
36. Cooper BA, Rosenblatt PS. Inherited defects of vitamin B₁₂ metabolism. *Ann Rev Nutr* 1987; **7**: 291.
37. Andersson HC, Shapira E. Biochemical and clinical response to hydroxocobalamin versus cyanocobalamin treatment in patients with methylmalonic acidemia and homocystinuria (cbl C). *J Pediatr* 1998; **132**: 121.
38. Tuchman M, Kelly P, Watkins D, Rosenblatt DS. Vitamin B₁₂-responsive megaloblastic anemia, homocystinuria, and transient methylmalonic aciduria in Cbl E disease. *J Pediatr* 1988; **113**: 1052.
39. Bodamer O. Creatine metabolism and Cbl C. *OAA Newsletter* 2002; **12**: 11.

Multiple carboxylase deficiency/holocarboxylase synthetase deficiency

Introduction	40	Treatment	44
Clinical abnormalities	41	References	45
Genetics and pathogenesis	42		

MAJOR PHENOTYPIC EXPRESSION

Erythematous, scaly eruption; alopecia; episodic, potentially lethal attacks of vomiting, ketosis, acidosis, and dehydration progressive to coma; lactic acidemia; organic aciduria including 3-methylcrotonylglycine, 3-hydroxyisovaleric acid, methylcitric acid, and 3-hydroxypropionic acid; defective activity of the propionyl CoA, 3-methylcrotonyl-CoA and pyruvate carboxylases; and defective activity of holocarboxylase synthetase ([Figure 5.1](#)).

INTRODUCTION

The first patient described with this disorder [1] was recognized as having an abnormality of leucine metabolism by the identification of 3-methylcrotonylglycine and 3-hydroxyisovaleric acid in the urine. When we found that methylcitric and hydroxypropionic acids were also excreted by the same patient [2], enzymatic analysis revealed defective activity of propionyl CoA carboxylases [3], as well as 3-methylcrotonyl CoA carboxylase [4]. The third

mitochondrial carboxylase, pyruvate carboxylase, was also shown to be defective in activity [5]. The disorder was then renamed 'multiple carboxylase deficiency' ([Figure 5.2](#)). It is now clear that there are two distinct disorders in which there is multiple carboxylase deficiency: holocarboxylase synthetase (HCS) deficiency ([Figure 5.1](#)) [6], which was the defect in the initial patient, and biotinidase deficiency ([Chapter 6](#)).

The gene for holocarboxylase synthetase has been cloned and assigned to chromosome 21q22 [7, 8]. The nature of

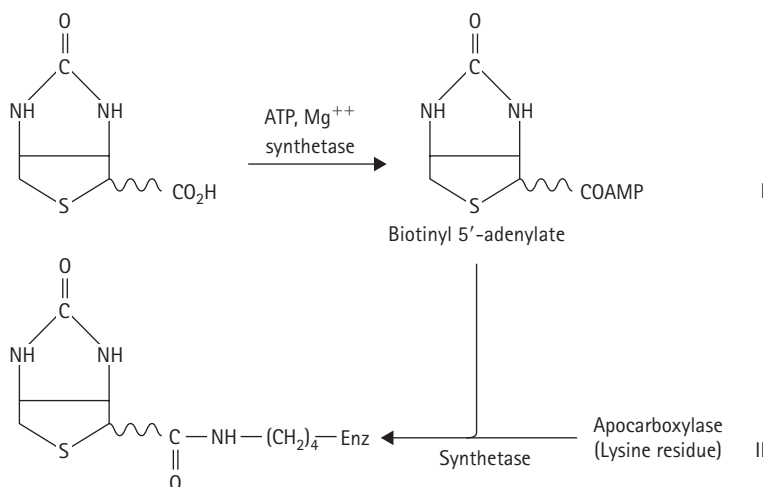


Figure 5.1 Holocarboxylase synthetase.

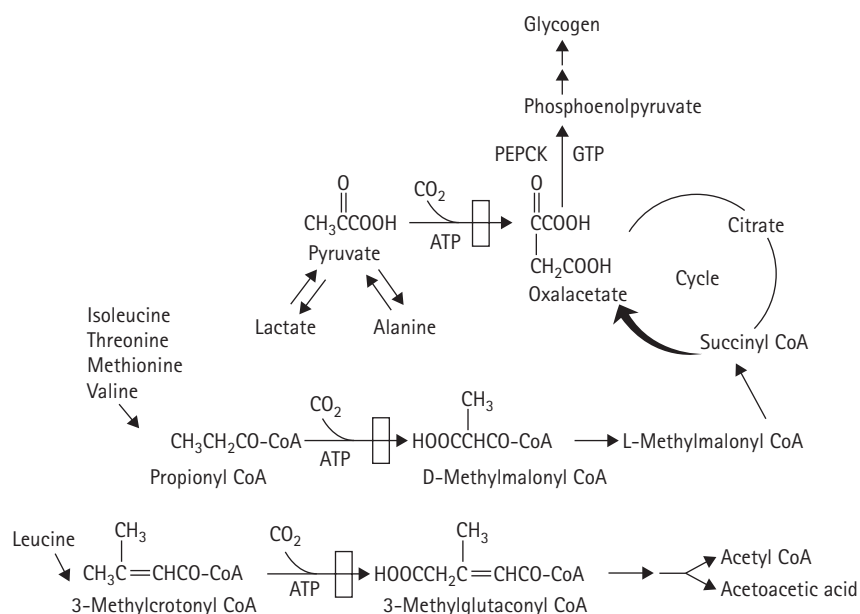


Figure 5.2 Pyruvate carboxylase, propionyl CoA carboxylase, and 3-methylcrotonyl carboxylase. The activities of each are deficient in multiple carboxylase deficiency.

the mutation was defined in two Japanese patients: a one-base deletion (1delG1067), which results in a premature termination; and a missense mutation (T997C), which changes a leucine to a proline. These mutations were found in a number of Japanese mutant alleles. Examination of European and Middle Eastern populations has revealed a variety of mutations, none of them common [9]. Expression yielded activity ranging from 1 to 14 percent of control. Among a variety of mutations reported, p.R508W and p.V550M were found in different ethnic groups and in different haplotypes, suggesting recurrent mutation [10]. Expression of the L237P mutation yielded an enzyme with decreased activity [9].

Among the most rewarding features of the disease is the exquisite sensitivity of most variant enzymes to treatment of the patient with biotin, which converts an otherwise uniformly fatal disease to completely normal health.

CLINICAL ABNORMALITIES

Patients with HCS deficiency generally present in the first days or months of life with overwhelming illness identical to those of propionic acidemia (Chapter 2) or other classic organic acidemia [1, 11–16]. In seven patients in whom the enzyme defect was documented [14], the age of onset of clinical symptoms varied from the first day of life to 18 months [17]. Most patients presented before 6 weeks of age, but it is clear that patients with an abnormal holocarboxylase synthetase can present at any age from 1 day to six years of age [18, 19]. The initial impression that the two forms of multiple carboxylase deficiency could be differentiated by the age of onset has not held up, although those with holocarboxylase synthetase abnormalities [20] have generally presented within the first 6 weeks of life,

while those with biotinidase deficiency have generally presented after six months of age.

In the acute episode of illness, the infant has massive ketosis and metabolic acidosis with an anion gap. There may be tachypnea or Kussmaul breathing. Concentrations of ammonia in the blood may be elevated. The episode may progress to dehydration, deep coma, and, unless vigorously treated, death. There is documentation of a number of patients who have died of this disease [11–21]. In fact, the initial episode may be lethal within hours of birth [11].

The classic patient with this disease was JR [1], in whom all the initial studies were performed [1–5, 22] and the defect in the HCS enzyme worked out [6, 14]. He had had recurrent episodes of vomiting from birth. An erythematous skin rash appeared at 6 weeks of age. At five months, he developed rapid respirations, vomiting, and unresponsiveness, and was found to have ketosis and metabolic acidosis.

The manifestations of the disease in the skin are memorable (Figure 5.3). An erythematous eruption usually involves the entire body. Some patients have died before the development of skin lesions, and now patients are being treated before the development of cutaneous lesions, but cutaneous features are an integral part of the untreated disease. The lesions are bright red, scaly, or desquamative. Intertriginous areas may be exudative. Complicating infection with monilia is common. The differential diagnosis of the skin disease includes acrodermatitis enteropathica, seborrheic dermatitis, and ichthyosis. The dermatosis is identical to that of clinical biotin deficiency [23]. Varying degrees of alopecia (Figure 5.4), an unusual manifestation in childhood, are associated, including alopecia totalis (Figure 5.4); eyelashes, eyebrows, and lanugo hair are absent, as well as the hair of the head. The differential diagnosis of alopecia includes:

- multiple carboxylase deficiency (HCS and biotinidase deficiencies)
- biotin deficiency
- cartilage hair hypoplasia
- an(hypo)hidrotic ectodermal dysplasia
- trichorhexis nodosa – argininosuccinic aciduria
- vitamin D receptor abnormalities.

Persistent vomiting may lead to failure to thrive. Neurologic abnormalities are not integral features of the disease; they appear to be related more to the effects of the initial, or repeated, episodes of illness in which there might be diminished perfusion of the brain or hyperammonemia, and the neurological examination may be normal despite a hyperammonemic episode [24]. Hypotonia has been observed, as well as hypertonia and irritability [25, 26]. Athetoid movements and opisthotonus have been described [27], as has 'cerebral palsy' [28]. There may be abnormalities of the electroencephalogram (EEG), and



Figure 5.3 AF at nine months of age. He had a bright red scaly eruption. Alopecia was not prominent during this relapse.



Figure 5.4 MZ at three months of age. The scaly eruption was erythematous and present throughout the body. He had almost complete alopecia of the scalp, except for a small amount of occipital hair, but there were sparse eyebrows and eyelashes.

abnormalities of computed tomography (CT) or magnetic resonance imaging (MRI) scans, particularly in the white matter. An infant was reported [29] to have subependymal cysts, seen on cranial ultrasound and MRI, which disappeared following six months of treatment with biotin. Subependymal cysts were also observed in seven Samoan infants who had severe disease, incomplete responsiveness to biotin, and early metabolic compensation and death [30]. Our patient with a poor dermatologic response to biotin (Figure 5.3) was Samoan; his sister died in the neonatal period [11].

Patients have disordered immunologic function of both T and B cells [11]. A diminution in the number of circulating T lymphocytes has been observed along with a diminution in their *in vitro* response to *Candida* in a patient with a history of bacteremia [25].

GENETICS AND PATHOGENESIS

The disorder is transmitted as an autosomal recessive trait. Both males and females are affected, and siblings of uninvolved parents have been observed. Consanguinity has been documented [1, 11, 26]. Fibroblast cultures from two unrelated individuals were studied using the complementation assay for propionic acidemia [5]. They failed to complement each other, but they did complement mutants for all of the other groups studied, such as propionic acidemia. Heterozygote detection has not been possible by enzyme analysis, but in a family in which the mutation is known it should not be demanding.

The metabolic hallmark of this disease is the excretion of 3-methylcrotonylglycine and 3-hydroxyisovaleric acid along with elevated amounts of lactic acid in the blood and urine. Thus, the first clinical chemical clue to the disease may be the documentation of lactic acidemia. Organic acid analysis at the time of acute acidosis also reveals methylcitric and 3-hydroxypropionic acids. The organic acidemia may be quite variable, particularly if first studied after intensive therapy with parenteral fluid and electrolytes and resolution of the acidosis. The excretion of 3-hydroxyisovaleric acid is virtually always greater than that of 3-methylcrotonylglycine [11, 20, 27]; but occasionally, the proportion of these values was reversed [1]. The excretion of 3-hydroxyisovaleric may be as high as 200 times normal [2]. The lactic aciduria may be enormous. These patients may also excrete tiglylglycine in the urine [11].

The lactic acidosis may be striking [15]. In an infant with lactic acidosis, it is important to consider this possibility and to assay the organic acids in the urine; if organic acid analysis is not promptly available, a trial of biotin therapy is warranted (with the urine saved for analysis).

The activities of the carboxylases (Figure 5.2) may be measured in leukocyte extracts or in fibroblasts, as well as in tissues. In patients with holocarboxylase synthetase deficiency, we have found levels of activity ranging from

Table 5.1 Carboxylase activity in holocarboxylase synthetase deficiency

	Normal range (pmol/min mg/protein)		Patient data (% of control)	
	Leukocyte	Fibroblast	Leukocyte	Fibroblast
Propionyl CoA carboxylase	160–447	128–537	12–43	0.7–52
3-Methylcrotonyl CoA carboxylase	62–288	71–250	15–34	0–29
Pyruvate carboxylase	7–14	96–362	29–53	2–60

0.4 to 53 percent of control (Table 5.1). In parallel studies of propionyl CoA and 3-methylcrotonyl CoA carboxylases in fibroblasts, their kinetic properties were normal [3]. The levels of activity of these enzymes are dependent on the concentrations of biotin in the medium. Activity of all of the carboxylases is markedly deficient when fibroblasts are grown in 6 nmol biotin/L. Carboxylases are normal when the cells are grown in 100 nmol biotin/L.

The fundamental defect is in holocarboxylase synthetase (EC 6.3.4.10) (Figure 5.1) [6, 14]. This is a complex enzyme which activates biotin to form D-biotinyl-5'-adenylate, and then catalyzes the attachment of the biotin to an ϵ -amino group of a lysine residue of the newly synthesized apocarboxylase enzyme. The covalent binding to biotin conveys enzymatic activity and holocarboxylase status to the apocarboxylase protein, which is inactive prior to this conversion.

To date, all but one patient studied has had altered K_m for biotin; the normal K_m is 1–6 nmol/L, and values in 16 patient cells ranged from 9 to 12 nmol/L. The maximum velocity (V_{max}) at saturation concentrations of biotin may be normal or reduced. There appears to be good correlation between the age of onset and severity of illness and chemical responsiveness to biotin and the degree of elevation of the K_m for biotin and residual activity of holocarboxylase synthetase [10, 14]. Our patient (Figure 5.3) with 70 times normal K_m for biotin presented in the first few hours of life [12], and a previous sibling had died in the neonatal period [11]. Patients in whom the K_m values for biotin and residence activity of holocarboxylase synthetase were 20–45 times normal presented between 1 day and 7 weeks of life. A patient with a K_m for biotin only three times normal presented at eight months of age. The K_m for biotin was not elevated in the enzyme coded for by the L237P mutation [9]; the V_{max} for this enzyme was 4.3 percent of the control mean.

The initial enzymatic step, the biotinyl-AMP (adenosine monophosphate) synthetase reaction, has now been found to be deficient in each of the fibroblast lines studied [31]. In four patients studied, activity ranged from 0.3 to 8 percent of controls. This makes for a much simpler assay than that currently available for the whole reaction, in which the substrate for the synthetase is an apocarboxylase carefully purified from the liver of biotin-deficient rats. Biotinylation has also been studied by reaction with p-67, a

peptide containing the last 67 amino acids of the α -subunit of propionylCoAcarboxylase followed by electrophoretic separation [32].

The cDNA for holocarboxylase synthetase [7, 33] codes for a protein of 726 amino acids [7, 31]; amino acids 445–701 have homology with related enzymes in *E. coli* and yeast. Among the mutations identified, a number in this domain are considered to bind biotin, for instance R508W, G518E, and V550M [34]. This is consistent with the clinical biotin responsiveness of six patients. However, patients with mutations outside this area, like those with the L237P mutation have also responded to biotin. L237P and c.780 delG were predominant only in Japanese patients and were thought to be founder mutations [10]. Similarly, c.1519 + 5G>A appears to be a founder mutation in Scandinavians; it is about ten times more prevalent in the Faroe Islands than in the rest of the world [10]. R508W was found in three late onset Chinese patients, two of them homozygous [35]. Neonatal onset has been found in patients with null mutations and point mutations associated with very little residual activity [10]. L216R was found in homozygosity in only one of the Samoan patients with very severe disease [30].

Prenatal diagnosis has been accomplished by the demonstration of biotin-responsive deficiencies of carboxylases in cultured amniocytes. It has also been demonstrated by assay of the activity of holocarboxylase synthetase [36]. Prenatal diagnosis has also been made by the direct assay of methylcitric acid in the amniotic fluid by stable isotope dilution and selected ion monitoring using gas chromatography-mass spectrometry (GCMS), as in propionic acidemia (Chapter 2). However, the concentrations of 3-hydroxyisovaleric acid in the amniotic fluid of patients with HCS deficiency are higher and its quantification is more reliable [37]. This is the best chemical method for the rapid prenatal diagnosis of this disorder and may unequivocally indicate an affected fetus. However, we have encountered the situation in which the results of this assay were equivocal, in which case assay of holocarboxylase synthetase gave the correct diagnosis [38]. Prenatal diagnosis has been carried out by enzyme assay of amniocytes [36, 38] and chorionic villus material [39]. In each case, a markedly elevated K_m for biotin was diagnostic. In a family in which the mutation is known, molecular methods may be used for prenatal diagnosis.

TREATMENT

Virtually all patients have been exquisitely sensitive to treatment with exogenous biotin. None have had acute attacks of ketoacidosis while taking biotin. The initial dose employed was 10 mg/day, and most patients have responded well to this dose. Nevertheless, heterogeneity in this condition may be manifest in the level of responsiveness to biotin. In some patients, small amounts of metabolites, present when the dose was 10 mg of biotin per day in the urine, disappeared when it was increased to 40 mg/day [25, 36]. Another patient, although clinically well when receiving as little as 1 mg of biotin per day, had elevated excretions of metabolites and activities of the carboxylases in leukocytes that were only 4–16 percent of normal when the dose was 20 mg of biotin per day [40]. A patient with a very high K_m for biotin [14] continued to have skin lesions, large excretions of metabolites and impaired activities of carboxylases in lymphocytes when receiving doses of biotin as high as 60 mg per day. Another patient required 100 mg per day before skin lesions resolved, and the relevant organic acids were present in small amounts in the urine, even on this regimen [18]. When provided with adequate amounts of biotin, none of the patients have required dietary restriction of protein, although moderate restriction in the less responsive patients could well decrease the accumulation of metabolites.

The clinical response to treatment is dramatic. Ketosis and acidosis disappear along with hyperammonemia. Levels of lactic and pyruvic acid in the blood become normal [41]. Lethargy, hypotonia, and ataxia disappear [2]. The skin lesions disappear in virtually all patients and the hair grows. Abnormalities of the EEG and CT scan have been documented to disappear [12]. At the same time, persisting neurologic abnormalities, such as developmental delay [18, 26] and dilated ventricles [12] once developed would not be expected to regress. The biochemical response to treatment is striking; the levels of organic acid metabolites often decrease to normal, but it may remain possible to continue to detect 3-hydroxyisovaleric acid in the urine [20, 23]. The activities of the carboxylases in leukocytes usually become normal within a few days of the initiation of therapy.

Few pharmacokinetic data have been assembled, but blood levels of biotin approximating 100 ng/mL have been reported in a three-month-old infant receiving 10 mg of biotin per day [42]. Urinary excretion varied between 2 and 4 mg/g creatinine. In a two-year-old child receiving the same dose, plasma levels as high as 703 ng/mL were observed [26], and in a neonate a level of 660 ng/mL was found following a dose of 20 mg per day [25]. These values exceed the range of the altered K_m for biotin in the patients studied and provide a potentially elegant correlation of the kinetics of each variant enzyme and the clinical pharmacology of biotin. A patient who responded nicely to 100 mg of biotin daily after a poor response to 10 mg/day achieved plasma concentrations of over 500 nM, demonstrably higher than the K_m of the enzyme for biotin



Figure 5.5 Prenatal diagnosis of holocarboxylase synthetase. JW, the boy on the left, presented with typical neonatal ketoacidosis, but was diagnosed promptly and treated with biotin and has not had a further episode. His sister, on the right, was diagnosed and treated prenatally and has never had symptoms of multiple carboxylase deficiency. The brother in the middle was normal.

of 164 nM [32]. Measurement of these pharmacokinetic data provides an elegant approach to the determinations of optimal treatment.

Prenatal therapy with biotin has been successfully pursued in at least four pregnancies at risk [36–39, 43]. In most, the affected fetus was diagnosed prenatally [36–39]. The dose of biotin to the mother was 10 mg/day, and levels of biotin in maternal serum were very high. There were no ill effects in the mother or fetus. At birth, assay of holocarboxylase synthetase in cultured skin fibroblasts indicated that infants were affected, but they were clinically well and had levels of urinary organic acids that were normal (Figure 5.5). Prenatal treatment in this condition appears prudent, because birth itself may be sufficiently catabolic that an affected infant may become irreversibly moribund within hours of birth [11]; and certainly death following ketoacidosis and disseminated intravascular coagulation has been recorded even after initiation of biotin therapy [44]. An infant, in whom treatment was carried out prenatally without diagnosis, then suspended while fibroblast cultures were established and an enzymatic diagnosis made, developed severe ketoacidosis, lactic acidemia and shock, but did respond to biotin therapy [43].

REFERENCES

1. Gompertz D, Draffan GH, Watts JL, Hull D. Biotin-responsive 3-methylcrotonylglycinuria. *Lancet* 1971; **2**: 22.
2. Sweetman L, Bates SP, Hull D, Nyhan WL. Propionyl-CoA carboxylase deficiency in a patient with biotin responsive 3-methylcrotonylglycinuria. *Pediatr Res* 1977; **11**: 1144.

3. Weyler W, Sweetman L, Maggio DC, Nyhan WL. Deficiency of propionyl-CoA carboxylase in a patient with methylcrotonylglycinuria. *Clin Chim Acta* 1977; **76**: 321.
4. Gompertz D, Goodey PA, Bartlett K. Evidence for the enzymatic defect in 3-methylcrotonylglycinuria. *FEBS Lett* 1973; **32**: 13.
5. Saunders M, Sweetman L, Robinson B *et al*. Biotin-responsive organic aciduria. Multiple carboxylase defects and complementation studies with propionic acidemia in cultured fibroblasts. *J Clin Invest* 1979; **64**: 1695.
6. Burri BJ, Sweetman L, Nyhan WL. Mutant holocarboxylase synthetase. Evidence for the enzyme defect in early infantile biotin-responsive multiple carboxylase deficiency. *J Clin Invest* 1981; **68**: 1491.
7. Suzuki Y, Aoki Y, Ishida Y *et al*. Isolation and characterization of mutations in the holocarboxylase synthetase cDNA. *Nature Genet* 1994; **8**: 122.
8. Zhang XX, Leon-Del-Rio A, Gravel RA, Eydoux P. Assignment of holocarboxylase synthetase gene (HLCS) to human chromosome band 21q22.1 and to mouse chromosome band 16C4 by *in situ* hybridization. *Cytogenet Cell Genet* 1997; **76**: 179.
9. Aoki Y, Li X, Sakamoto O *et al*. Identification and characterization of mutations in patients with holocarboxylase synthetase deficiency. *Hum Genet* 1999; **104**: 1443.
10. Suzuki Y, Yang X, Aoki Y *et al*. Mutations in the holocarboxylase synthetase gene HLCS. *Hum Mutat* 2005; **26**: 285.
11. Sweetman L, Nyhan WL, Sakati NA *et al*. Organic aciduria in neonatal multiple carboxylase deficiency. *J Inherit Metab Dis* 1982; **5**: 49.
12. Wolf B, Hsia E, Sweetman L *et al*. Multiple carboxylase deficiency: clinical and biochemical improvement following neonatal biotin treatment. *Pediatrics* 1981; **68**: 113.
13. Bartlett K, Ng H, Dale G *et al*. Studies on cultured fibroblasts from patients with defects of biotin-dependent carboxylation. *J Inherit Metab Dis* 1981; **4**: 183.
14. Burri BJ, Sweetman L, Nyhan WL. Heterogeneity of holocarboxylase synthetase in patients with biotin-responsive multiple carboxylase deficiency. *Am J Hum Genet* 1985; **37**: 326.
15. Briones P, Ribes A, Vilaseca MA *et al*. A new case of holocarboxylase synthetase deficiency. *J Inherit Metab Dis* 1989; **12**: 329.
16. Michalski AJ, Berry GT, Segal S. Holo-carboxylase synthetase deficiency: 9-year follow-up of a patient on chronic biotin therapy and a review of the literature. *J Inherit Metab Dis* 1989; **12**: 312.
17. Suormala T, Fowler B, Jakobs C *et al*. Late-onset holocarboxylase synthetase-deficiency: Pre- and post-natal diagnosis and evaluation of effectiveness of antenatal biotin therapy. *Eur J Pediatr* 1998; **157**: 570.
18. Suormala T, Fowler B, Duran M *et al*. Five patients with a biotin-responsive defect in holocarboxylase formation: Evaluation of responsiveness to biotin therapy *in vivo* and comparative biochemical studies *in vitro*. *Pediatr Res* 1997; **41**: 666.
19. Sherwood WG, Saunders M, Robinson BH *et al*. Lactic acidosis in biotin-responsive multiple carboxylase deficiency caused by holocarboxylase synthetase deficiency of early and late onset. *J Pediatr* 1982; **101**: 546.
20. Sweetman L. Two forms of biotin-responsive multiple carboxylase deficiency. *J Inherit Metab Dis* 1981; **4**: 53.
21. Roth K, Cohn R, Yandrasitz J *et al*. Beta-methylcrotonic aciduria associated with lactic acidosis. *J Pediatr* 1976; **88**: 229.
22. Gompertz D, Draffan GH. The identification of tiglylglycine in the urine of a child with 3-methylcrotonyl-glycinuria. *Clin Chim Acta* 1972; **37**: 405.
23. Sweetman L, Surh L, Baker H *et al*. Clinical and metabolic abnormalities in a boy with dietary deficiency of biotin. *Pediatrics* 1981; **68**: 553.
24. Dabbagh O, Brismar J, Gascon GG, Ozand PT. The clinical spectrum of biotin-treatable encephalopathies in Saudi Arabia. *Brain Dev* 1994; **16**(Suppl.): 72.
25. Packman S, Sweetman L, Baker H, Wall S. The neonatal form of biotin-responsive multiple carboxylase deficiency. *J Pediatr* 1981; **99**: 418.
26. Leonard JV, Seakins JWT, Bartlett K *et al*. Inherited disorders of 3-methylcrotonyl CoA carboxylation. *Arch Dis Child* 1981; **56**: 53.
27. Gompertz D, Bartlett K, Blair D, Stern CMM. Child with a defect in leucine metabolism associated with 3-hydroxyisovaleric aciduria and 3-methylcrotonylglycinuria. *Arch Dis Child* 1973; **48**: 975.
28. Livne M, Gibson KM, Amir N *et al*. Holocarboxylase synthetase deficiency: A treatable metabolic disorder masquerading as cerebral palsy. *J Child Neurol* 1994; **9**: 170.
29. Squires L, Betz B, Umfleet J, Kelley R. Resolution of subependymal cysts in neonatal holocarboxylase synthetase deficiency. *Dev Med Child Neurol* 1997; **39**: 267.
30. Wilson CJ, Myer M, Darlow BA *et al*. Severe holocarboxylase synthetase deficiency with incomplete biotin responsiveness resulting in antenatal insult in Samoan neonates. *J Pediatr* 2005; **147**: 115.
31. Morita J, Thuy LP, Sweetman L. Deficiency of biotinyl-AMP synthetase activity in fibroblasts of patients with holocarboxylase synthetase deficiency. *Mol Genet Metab* 1989; **64**: 250.
32. Van Hove JLK, Josefsbert S, Freehauf C *et al*. Management of a patient with holocarboxylase synthetase deficiency. *Mol Genet Metab* 2008; **95**: 201.
33. Leon-Del-Rio A, Leclerc D, Akerman B *et al*. Isolation of a cDNA encoding human holocarboxylase synthetase by functional complementation of a biotin auxotroph of *Escherichia coli*. *Proc Natl Acad Sci USA* 1995; **92**: 4626.
34. Dupuis L, Leon-Del-Rio A, Leclerc D *et al*. Clustering of mutations in the biotin-binding region of holocarboxylase synthetase in biotin-responsive multiple carboxylase deficiency. *Hum Mol Genet* 1996; **5**: 1011.
35. Tang NL, Hui J, Yong CK *et al*. A genomic approach to mutation analysis of holocarboxylase synthetase gene in three Chinese patients with late-onset holocarboxylase synthetase deficiency. *Clin Biol Chem* 2003; **36**: 145.
36. Packman S, Cowan MJ, Golbus MS *et al*. Prenatal treatment of biotin-responsive multiple carboxylase deficiency. *Lancet* 1982; **1**: 1435.

37. Jakobs C, Sweetman L, Nyhan WL, Packman S. Stable isotope dilution analysis of 3-hydroxyisovaleric acid in amniotic fluid: contribution to the prenatal diagnosis of inherited disorders of leucine catabolism. *J Inherit Metab Dis* 1984; **7**: 15.
38. Thuy LP, Belmont J, Nyhan WL. Prenatal diagnosis and treatment of holocarboxylase synthetase deficiency. *Prenat Diagn* 1999; **19**: 108.
39. Thuy LP, Jurecki E, Nemzer L, Nyhan WL. Prenatal diagnosis of holocarboxylase synthetase deficiency by assay of the enzyme in chorionic villus material followed by prenatal treatment. *Clin Chim Acta* 1999; **284**: 59.
40. Narisawa K, Arai N, Igarashi Y *et al*. Clinical and biochemical findings on a child with multiple biotin-responsive carboxylase deficiencies. *J Inherit Metab Dis* 1982; **5**: 67.
41. Charles BM, Hosking G, Green A *et al*. Biotin-responsive alopecia and developmental regression. *Lancet* 1979; **2**: 188.
42. Gaudry M, Munnich A, Ogier H *et al*. Deficient liver biotinidase activity in multiple carboxylase deficiency. *Lancet* 1983; **2**: 397.
43. Roth KS, Yang W, Allan L *et al*. Prenatal administration of biotin in biotin-responsive multiple carboxylase deficiency. *Pediatr Res* 1982; **16**: 126.
44. Roth KS, Yang W, Foreman JW *et al*. Holocarboxylase synthetase deficiency: a biotin-responsive organic acidemia. *J Pediatr* 1980; **96**: 845.

Multiple carboxylase deficiency/biotinidase deficiency

Introduction	47	Treatment	54
Clinical abnormalities	48	References	54
Genetics and pathogenesis	52		

MAJOR PHENOTYPIC EXPRESSION

Seizures, ataxia, hypotonia, alopecia, periorificial cutaneous eruption, episodic metabolic acidosis, hearing loss, loss of vision, developmental delay; lactic acidemia, propionic acidemia, excretion of 3-methylcrotonylglycine, 3-hydroxyisovaleric acid, methylcitric acid, and 3-hydroxypropionic acid in urine; and defective activity of biotinidase.

INTRODUCTION

Biotinidase deficiency is a form of multiple carboxylase deficiency in which the fundamental defect is an inability to cleave biocytin (Figures 6.1 and 6.2), and this leads to defective activity of propionylCoA carboxylase, 3-methylcrotonylCoA carboxylase, and pyruvate

carboxylase [1]. Multiple carboxylase deficiency is also caused by defective activity of holocarboxylase synthetase (Chapter 5) [2]. In earlier literature, biotinidase deficiency was referred to as the later infantile form of multiple carboxylase deficiency [1, 3] to distinguish it from the usual neonatal presentation of holocarboxylase synthetase deficiency. However, it is now clear that the latter disorder

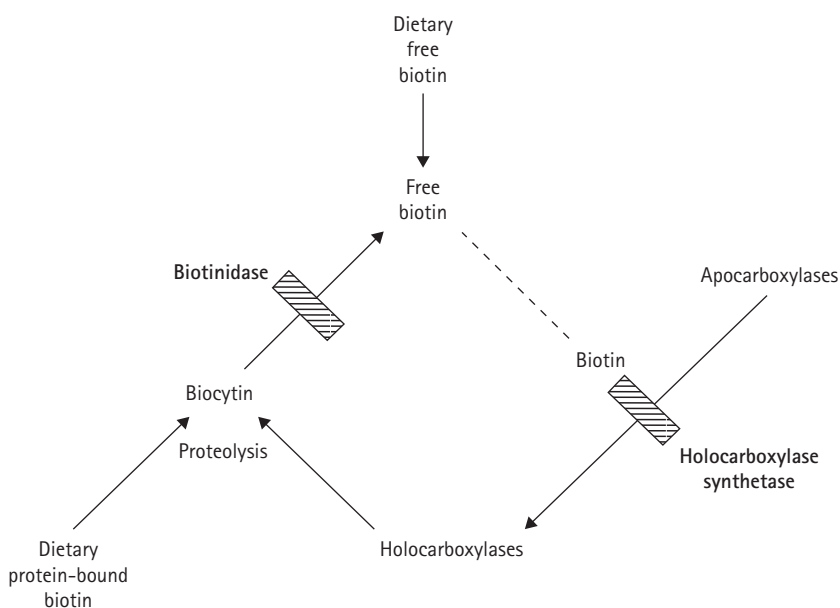


Figure 6.1 Pathways of metabolism of biotin. Biotin is an essential cofactor for all carboxylase enzymes. Attachment to the inactive newly synthesized apocarboxylase is catalyzed by holocarboxylase synthetase. Biotin is recycled through the activity of biotinidase, and this enzyme would also be required to release biotin bound to protein in the intestine.

can present later and the former earlier; the way to distinguish them unambiguously is by enzyme analysis or determination of mutation.

Biotin, as a vitamin, cannot be synthesized by humans, but in addition to dietary sources, it is synthesized by intestinal microflora. There are dietary sources of free biotin, but covalently bound biotin must ultimately be acted upon by biotinidase to make biotin available from either dietary, intestinal bacterial or recycled sources (Figures 6.1 and 6.2). Biotin is an intrinsic cofactor for each of the carboxylase enzymes, which are synthesized as inactive apoenzymes and must be linked with biotin in the holocarboxylase synthetase reaction (Chapter 5) to become active holoenzymes.

The cDNA for biotinidase has been cloned [4], and the gene has been mapped to chromosome 3p25 [5]. At least 21 mutations were found [6] in 37 children with profound, symptomatic deficiency of biotin. Two were common, accounting for over half of the alleles studied, one a deletion/insertion delG98-G104: insTCC (G98:d7i3) which results in a frame shift and premature termination and the other R538C, a substitution of T for C at nucleotide 1612, only five amino acids from the carboxy terminus of the enzyme. Variants with partial deficiency of biotinidase deficiency have the mutation D444H, in compound with a mutation causing profound deficiency when homozygous on the other allele [7, 8].

Most of the clinical abnormalities of biotinidase deficiency respond well to relatively small doses of biotin. However, optic atrophy, hearing loss, and spastic diplegia once developed do not resolve. In a series of 20 Turkish patients [9], all of those with hearing loss had null mutations, but three children ascertained and treated early because of an affected sibling had normal hearing despite null mutation. This provides further argument for newborn screening. It also provides argument for mutational analysis in the counseling of families.

Biotinidase deficiency presents with a median age of three months or as late as ten years of age. Symptoms may begin in the neonatal period. Initial symptoms may be dermatologic [10] or seizures [11]. Early infantile seizures may be myoclonic.



Figure 6.3 FE: A 27-month-old Saudi Arabian girl with biotinidase deficiency. She lost all scalp hair and eyebrows at 20 days, but it returned; scalp hair disappeared at eight months. In addition, she had reddened dermatitis about the eyes and mouth, with cracking at the corners of the lips.



Figure 6.4 FE: There was also extensive perineal dermatitis. In addition, she had spastic quadripareisis.



Figure 6.5 FE: After six months of treatment with biotin. She had lost the spastic quadriplegia, had no dermatitis and abundant hair.



Figure 6.7 RR: A girl with biotinidase deficiency, illustrating the periorificial lesions that led to a diagnosis of acrodermatitis enteropathica. The hair was sparse. (Illustration was kindly provided by Dr Seymour Packman, University of California, San Francisco.)



Figure 6.6 FE: Six months later, illustrating the return of the alopecia following noncompliance with biotin therapy. She again responded to treatment, but had essentially total loss of hearing.

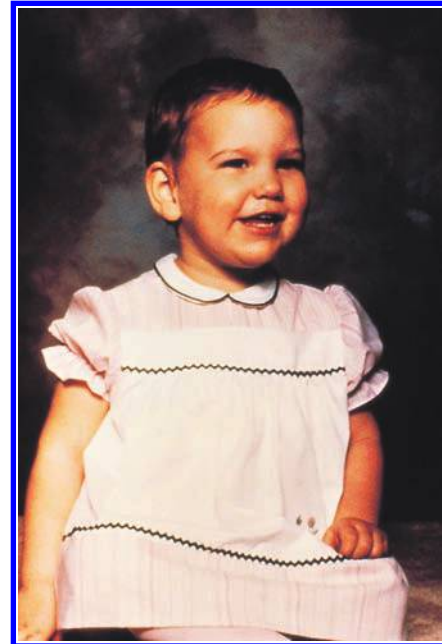


Figure 6.8 RR: Illustrating the response to treatment with biotin. (Illustration was kindly provided by Dr Seymour Packman, University of California, San Francisco.)

The cutaneous lesions tend to be patchy [11–15] (Figures 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 6.10, and 6.11), in contrast to the total body eruption seen in holocarboxylase synthetase deficiency. However, there may be severe generalized involvement of the skin with redness and desquamation

[13]. Skin lesions are associated with periorificial cracking, and there may be blepharoconjunctivitis or keratoconjunctivitis of sufficient severity to lead to admission to hospital. Corneal ulceration may occur.



Figure 6.9 JRo: A patient with biotinidase deficiency. In addition to the skin lesions and the alopecia, incapacitating neurologic disease made her bedridden. (Illustration was kindly provided by Dr Jess Thoene.)



Figure 6.10 CG: A boy with biotinidase deficiency, illustrating the characteristic lesions about the mouth and eyes and the alopecia. (Illustration kindly provided by Dr E Zammarchi, Dipartimento di Pediatria, Clinica Pediatrica 1, University of Florence, Florence, Italy.)



Figure 6.11 CG: Illustrating the complete reversal of the cutaneous lesions and the alopecia after treatment with biotin. He had significant hearing loss and pale optic discs. (Illustration kindly provided by Dr E Zammarchi, Dipartimento di Pediatria, Clinica Pediatrica 1, University of Florence, Florence, Italy.)

Perioral stomatitis is regularly seen, and there may be glossitis. There may also be perineal dermatitis (Figure 6.4) [13]. One of our patients had carried a clinical diagnosis of acrodermatitis enteropathica for many years. Anhidrotic ectodermal dysplasia has also been considered in the differential diagnosis of this disorder. The eruption may appear seborrheic. Mucocutaneous candidiasis is a frequent concomitant. The alopecia may be progressive to alopecia totalis (Figure 6.10) [11], but it is usually less than total (Figures 6.7 and 6.11), and may be simply a sparseness of cranial hair, eyebrows, or lashes.

In initial experience, the diagnosis was made in each patient because of the occurrence of typical episodes of acidosis, ketosis, and organic aciduria [3]. Severe, life-threatening acidosis may be seen [14], along with coma, hypothermia, massive hypotonia, and absent reflexes [16]. There may be chronic compensated acidosis with serum concentrations of bicarbonate in the range of 15 mEq/L [11]. Episodic acidosis may be seen at times of acute infection [12].

Neurological manifestations are major features of biotinidase deficiency. Ataxia is a prominent feature and may be so profound as to interfere with walking [11]. Ataxia may also be intermittent [15]. There may be associated intention tremor. Seizures occur in over 70 percent of patients and may be the only obvious symptom [17, 18]; so testing for biotinidase deficiency is warranted in any patient with unexplained seizures. Seizures may be generalized or myoclonic. They may be frequent, or intermittent, or they may occur only with fever. Infantile spasms may be the initial presenting feature of the disease [19]. Experience with this patient emphasized that an early neurologic presentation may lack any of the characteristic findings in the skin and hair. In one study [18] of 78 children, 55 percent had seizures, and of these, seizures were the presenting complaint in 70 percent. Seizures were

poorly controlled with anticonvulsant medication in 40 percent, but in 75 percent they disappeared after treatment with biotin. This experience, like other responses to biotin in multiple carboxylase deficiencies, can be one of the most striking and rewarding in medicine. Development may be delayed [11, 13]. Hypotonia has also been observed in over half of the patients [3]. Two patients developed acute severe hypotonia at ten months in which there was loss of head control [20, 21]. The neurologic degenerative picture of Leigh syndrome has been described in a number of patients [13, 22–24].

Stridorous or labored breathing and apnea have been seen in these patients [22], followed by psychomotor regression or bulbar symptoms [23]. Laryngeal stridor, a presenting feature in some patients [24, 25] has been interpreted as of neurologic in origin, and it has resolved with treatment with biotin [25]. Deep tendon reflexes may be brisk. Death from the disease has been reported at nine months and at three years of age [15].

Neurosensory abnormalities involving the optic and auditory nerves have been observed in a considerable number of patients, often as late manifestations [3, 26–31]. Loss of visual function is associated with optic atrophy [3, 26, 30]. It appears to be more common in patients in whom diagnosis and treatment is delayed [32]. Neurosensory hearing loss seems to follow the same pattern [3, 27]. Among 33 children diagnosed because of symptoms, and none treated from birth, 76 percent had hearing loss [33]. A patient diagnosed at ten months of age and treated with 10 mg of biotin per day [20, 28] subsequently developed sensorineural hearing loss and severe myopia with progressive retinal epithelial dysplasia and optic atrophy. Treatment with biotin does not necessarily prevent these optic and auditory manifestations, but the authors of the report [33] were not aware of hearing loss in any infants diagnosed via newborn screening and treated from the neonatal period. Many of the neurological features of disease disappear in response to treatment with biotin, as do the cutaneous and metabolic features; but sensorineural abnormalities involving the optic and auditory nerves are persistent. Since most of these patients have been treated for some time before these lesions are detected, the question has been raised that they might be a consequence of treatment with biotin; however, this seems unlikely because these complications have never been encountered in patients with holocarboxylase synthetase deficiency who have been treated with biotin from an earlier age, sometimes with higher doses. Furthermore, these abnormalities have been observed in a number of patients prior to the initiation of treatment with biotin [28, 33, 34]. One patient who presented and was diagnosed at five years of age had already developed sensorineural abnormalities of the optic and auditory nerves, which did not resolve with treatment [33].

An unusual late-onset presentation has been described [29, 35–37] in patients with spastic paraparesis studied at 13 and 15 years of age (Figure 6.12). In each, there was progressive optic atrophy. In one boy, the first symptom was



Figure 6.12 FM: An 18-year-old man with biotinidase deficiency who presented at 13 years with spastic diplegia and loss of vision. Vision improved with biotin, but optic discs were white and he remained wheelchair-dependent.

acute loss of vision during an intercurrent infection [33]. In this patient, there was improvement in visual acuity and disappearance of pyramidal tract signs in the lower limbs after months of treatment with biotin. In the other [29], there was considerable improvement in both areas, but he had spasticity and essentially wheelchair-bound. Acute loss of vision, optic atrophy, and spastic paraparesis developed in one patient at ten years of age [36]. In the Turkish series of patients [9] with profound deficiency of biotinidase, 55 percent had hearing loss.

Immunodeficiency has been reported [14] and there have been abnormalities in the function of both T and B cells. In two patients with extensive chronic mucocutaneous candidiasis, responses to *Candida* antigen *in vitro* and *in vivo* were absent. In one, there was a deficiency of IgA and no antibody response to immunization with pneumococcal polysaccharide; in the other, the percentage of circulating T lymphocytes was abnormally low. In this family, two previous siblings had died at eight and 39 months of age of what appeared to be the same disease [14, 38]. An unrelated patient [39] was reported to have impaired lymphocyte-suppressing activity *in vitro* that improved on treatment with biotin and fatty acids. Deficiency of biotin in guinea pigs has been associated with decreased numbers of T and B lymphocytes [40]. All of these immunologic problems disappear with biotin treatment. One patient was initially thought to have severe combined immunodeficiency and treated with bone marrow transplantation, but manifestations persisted until treatment with biotin was initiated [41].

The electroencephalograph (EEG) is often normal in biotinidase deficiency, but among children with seizures there were 16 abnormal EEGs [18]. Diffuse slowing or convulsive activity has been observed, and usually the EEG has rapidly become normal with biotin treatment [42]. Visual evoked potentials (responses) (VEP, VER) have been abnormal in a number of patients [43, 44], returning promptly to normal with biotin treatment. In a 13-year-old boy with optic atrophy and spastic paraparesis [29], positron emission tomography (PET) showed a low relative metabolic rate for glucose in temporal and occipital lobes, which became normal following treatment.

Neuroimaging studies have been highly variable. Calcification of the basal ganglia was reported in a 26-month-old infant [45]. Magnetic resonance imaging (MRI) and computed tomography (CT) evidence of white matter lucency indicative of delayed myelination and diffuse atrophy have been reported [46, 47].

The neuropathology has been characterized by atrophy and neuronal loss in the cerebellum [14]. Atrophy of the superior vermis has been associated with virtually complete disappearance of the layer of Purkinje cells. In addition, there was moderate gliosis in the white matter and a subacute necrotizing myelopathy. The histopathologic picture of subacute necrotizing encephalopathy has been reported in a patient with the clinical picture of Leigh syndrome [23]. There was rarefaction and spongy degeneration in the subcortical white matter, the midbrain, pons, and medulla.

The advent of newborn screening for biotinidase deficiency has raised the possibility that some untreated patients may be asymptomatic. Some with partial deficiency have been asymptomatic until stressed by significant infection [8]. On the other hand, unrelated asymptomatic adults have been diagnosed because their children failed newborn screening [48].

Newborn screening identifies infants with profound deficiency of biotinidase, defined as activity less than 10 percent of control mean; it also identifies those with partial deficiency (10–30 percent of control). Most, but not all, of those in the partial group have not developed clinical manifestations of disease.

GENETICS AND PATHOGENESIS

Biotinidase deficiency is transmitted in an autosomal recessive pattern. Siblings of uninvolved parents have been observed and consanguinity has been documented [16, 21]. Parents of patients display about 50 percent of normal activity of biotinidase consistent with heterozygosity [49]. Biotinidase activity is detectable in normal amniocytes, so that prenatal diagnosis of biotinidase deficiency should be possible, but this has not yet been reported, although prenatal assay of the enzyme in amniocytes and chorionic villi has yielded evidence of normal fetuses and a heterozygote [50, 51]. In a family in which the mutation

is known, molecular analysis is the method of choice for prenatal diagnosis and carrier detection.

Metabolic abnormalities in biotinidase deficiency include lactic acidemia, in the presence or absence of recurrent episodes of ketoacidosis. For this reason, testing for biotinidase deficiency is of interest in any patient with unexplained lactic acidemia. In young infants, there may be hyperammonemia during acute episodes of illness. The characteristic organic aciduria consists of the excretion of 3-methylcrotonylglycine, 3-hydroxyisovaleric acid, 3-hydroxypropionic acid, and 2-methylcitric acid [3, 10]. Some patients with otherwise typical phenotypes and enzyme deficiency have been reported not to have organic aciduria [3, 52, 53]. Elevated concentrations of lactate and pyruvate have been reported in the cerebrospinal fluid (CSF) [54], as was reversion to normal with treatment. In fact, an elevated cerebrospinal fluid concentration of lactate may occur in the absence of hyperlactic acidemia [23, 42, 54, 55]. The CSF to plasma ratio of lactic acid may be as high as 3.7 [55]. The concentration of 3-hydroxyisovaleric acid may also be higher in CSF than in plasma [55].

The diagnosis is made by the assay of biotinidase (Figure 6.2) in serum [1, 49]. The enzyme has also been shown to be deficient in the liver. Methods available for biotinidase include the cleavage of N-biotinyl-3-aminobenzoate [49], of biotinyl-¹⁴C-*p*-aminobenzoic acid [56] or of the ¹⁴C-labeled natural substrate N-biotinyllysine [57] and a sensitive fluorimetric method with biotinyl-6-aminoquinoline [58], which has advantages for kinetic and other studies over the release of *p*-aminobenzoate followed by diazotization in the Bratton–Marshall reaction. Values obtained with the various assays have been quite similar. In the original assay [3, 49] the mean was 5.80 and the range 4.30–7.54 nmol/min/mL, whereas values obtained in patients ranged from undetectable to 0.18 nmol/min/mL. Small amounts of biotinidase activity are detectable in normal fibroblasts, while none was found in patient fibroblasts.

Wolf and colleagues [59] have developed a simple colorimetric test for biotinidase deficiency that can be employed with spots of blood dried on filter paper, and they have demonstrated that the test is suitable for incorporation into a statewide program of neonatal screening. Two infants with the disease were identified among the first 81,243 infants screened. This led to the discovery of two more patients who were previously undiagnosed siblings. By now millions of newborns have been screened throughout the world and the yield of known patients has resulted in an estimate of frequency of biotinidase deficiency of one in 60,000 births [60].

Patients have been classified as profoundly or partially deficient on the basis of phenotype, the presence or absence of immunoreactive enzyme – cross-reacting material (CRM) – and the isoform pattern of sodium dodecylsulfate (SDS) immunoblots [61]. Most patients were CRM-positive and had normal kinetics of the enzyme. In one variant patient with late onset disease [36], the plasma biotinidase

enzyme displayed biphasic kinetics with two different low values for V_{max} and two K_m values. Among patients with profound deficiency of biotinidase, no relationship could be discerned between either age at onset or severity of symptom and the CRM status or isoform patterns [61]. On the other hand, it is clear that there is a population of patients with partial deficiency identified by newborn screening who are biochemically different from those who have come to attention because of symptomatic disease.

The cloning of the gene for biotinidase and the identification of the nature of mutation has brought better correlation of phenotype with genotype. The relatively common R538C mutation involves a CpG dinucleotide, a likely place for mutation [6], and this and the G98:d7i3 cause severe disease. On the other hand, compounds of the D444H with a variety of mutations including T404I and C594delC are associated with partial deficiency [62]. Patients with late onset disease, spastic paresis and optic problems had mutations L215F, R538C, V457M, and G98:d7i3, which are usually found in infantile onset severe disease [37]. T171G mutation has been observed only in Turkish populations [63]. In this population, many patients are homozygous for null mutations such as this one [9], and in this population all of those with null mutations had hearing loss, except for those treated early because of an affected sibling. On the other hand, all six children homozygous for missense mutations had normal hearing. The authors pointed out the advantages of homozygosity for making genotype phenotype correlation. Correlation of mutation with enzyme activity was less good and they attributed the detectable activity in patients with null mutations to variability in measuring activity of the enzyme.

The immediate consequence of biotinidase deficiency is that levels of biotin in blood, urine, and tissues are low [11]. The low level may be more dramatically evident in the urine than in the blood. These observations initially suggested a defect in the absorption or transport of biotin [64] or its excessive renal excretion.

Biocytin has been detected in the urine of patients with biotinidase deficiency [65]. This compound is not detectable in normal urine. The levels of biocytin were considerably higher than the levels of biotin when the patients were not being treated with biotin. If the normal renal clearance of biotin at half that of creatinine is a reflection of renal reabsorption, the increased clearance of biotin in biotinidase deficiency might be caused by an inhibition of biotin reabsorption by the elevated amounts of biocytin. Biocytin could compete with biotin as substrate for holocarboxylase synthetase, increasing the concentration of biotin needed for effective holocarboxylase synthesis. It is also possible that elevated levels of biocytin are directly toxic, but there is no evidence for this as yet. A specific high performance liquid chromatography (HPLC) method for biocytin has been developed [66]. In patients, prior to treatment, levels of biocytin in urine ranged from 6.2 to 28.8 nmol/mmol creatinine. During therapy, levels

increased 1.3- to 4-fold, but increase in dosage to 200 mg/day in a patient did not change the excretion of biocytin from the level observed with 10 mg/day. Other derivatives of biotin were found in the urine and tentatively identified as bis-norbiotin and oxidation products.

The low tissue stores of biotin that result from biotinidase deficiency led to deficient activity of carboxylases (see Figure 5.2), and this of course results in the lactic acidemia and the accumulation of the other organic acid metabolites. Activities of carboxylases were found to be more severely compromised in brain than in liver and kidney [23], which would be consistent with the higher levels of lactate and 3-hydroxyisovalerate found in CSF.

Activities of carboxylases in freshly isolated lymphocytes are low. On the other hand, activities of each of the carboxylases in cultured fibroblasts are normal whether cells are cultivated in high or low concentrations of biotin [12, 67–69]. This is typical of biotinidase deficiency and was used to distinguish these patients from those with holocarboxylase synthetase deficiency in whom fibroblasts display lower activity of carboxylases when grown in media containing low concentrations of biotin [70]. A rapid diagnostic method for distinguishing holocarboxylase synthetase abnormalities from biotinidase deficiency is the assay of the activity of carboxylases in freshly isolated lymphocytes in the presence and absence of preincubation with biotin [69]. Of course, direct assay of the relevant enzyme is diagnostic. Activity of holocarboxylase synthetase is normal in patients with biotinidase deficiency.

Biotinidase in human and rat brain is much lower than in other tissues, and biotin levels in brain are depleted in biotinidase deficiency earlier and more severely than in other tissues [23, 53]. This inefficient recycling of biotin would make the brain more dependent on transfer of biotin than other tissues, and thus more susceptible to deficiency. This would be consistent with the preferential elevation of lactate and 3-hydroxyisovalerate in the CSF and the occasional absence of organic aciduria, as well as the concomitance of CSF accumulation in patients with neurological symptoms. Pyruvate carboxylase may be predominantly affected by biotinidase deficiency in the brain. Neurological symptomatology could be the result of the toxic effect of local accumulation of lactate and organic acids. These observations are consistent with the fact that sometimes abnormalities of the central nervous system are the first manifestations of the disease. It is of interest that high CSF concentrations of lactate and 3-hydroxyisovalerate are not seen in holocarboxylase synthetase deficiency, where central nervous system abnormalities are not expected, except as a result of a catastrophic event. Concentrations of 3-hydroxyisovalerate in the CSF were high in a patient with isolated deficiency of 3-methylcrotonyl CoA carboxylase, and this patient had severe neurologic abnormalities, indicating that this compound, as well as lactate, may be toxic to brain.

TREATMENT

Patients are effectively treated with relatively small doses of biotin. The dose most commonly employed is 10 mg/day, but as little as 5 mg/day has been effective [12]. The organic aciduria and virtually all of the clinical manifestations of the disease disappear promptly after the initiation of treatment. However, auditory and optic nerve losses are not reversed [11, 14, 18, 27, 37, 71, 72]. Presymptomatic treatment in a patient diagnosed by assay of cord blood, because the disease had previously been diagnosed in a sibling, has been followed by completely normal development, including vision and hearing, for 14 months at report [73]. In one patient, oral and cutaneous administration of unsaturated fatty acids was followed by remission of alopecia and cutaneous lesions [74], suggesting that a deficiency of acetyl CoA carboxylase required for fatty acid synthesis is involved in the pathogenesis of these manifestations.

REFERENCES

1. Wolf B, Grier RE, Parker WD *et al.* Deficient biotinidase activity in late onset multiple carboxylase deficiency. *N Engl J Med* 1983; **308**: 161.
2. Burri BJ, Sweetman L, Nyhan WL. Mutant holocarboxylase synthetase: evidence for the enzyme defect in early infantile biotin-responsive multiple carboxylase deficiency. *J Clin Invest* 1981; **68**: 1491.
3. Wolf B, Grier RE, Allen RJ *et al.* Phenotypic variation in biotinidase deficiency. *J Pediatr* 1983; **103**: 233.
4. Cole H, Reynolds TR, Lockyer J *et al.* Human serum biotinidase: cDNA cloning sequence and characterization. *J Biol Chem* 1994; **269**: 6566.
5. Cole H, Weremowicz H, Morton CC, Wolf B. Localization of serum biotinidase (BTD) to human chromosome 3 in band p25. *Genomics* 1994; **22**: 662.
6. Pomponio RJ, Hymes J, Reynolds TR *et al.* Mutations in the human biotinidase gene that cause profound biotinidase deficiency in symptomatic children: molecular biochemical and clinical analysis. *Pediatr Res* 1997; **42**: 840.
7. Norrgard KJ, Pomponio RJ, Swango KL *et al.* Double mutation (A171T and D444H) is a common cause of profound biotinidase deficiency in children ascertained by newborn screening in United States. *Hum Mutat* 1998; **11**: 410.
8. Swango KL, Demirkol M, Huner G *et al.* Partial biotinidase deficiency is usually due to the D444H mutation in the biotinidase gene. *Hum Genet* 1998; **102**: 571.
9. Sivri HSK, Genc GA, Tokatli A *et al.* Hearing loss in biotinidase deficiency: genotype-phenotype correlation. *J Pediatr* 2007; **150**: 439.
10. Sweetman L. Two forms of biotin-responsive multiple carboxylase deficiency. *J Inherit Metab Dis* 1981; **4**: 53.
11. Thoene J, Baker H, Yoshino M, Sweetman L. Biotin-responsive carboxylase deficiency associated with subnormal plasma and urinary biotin. *N Engl J Med* 1981; **304**: 817.
12. Bartlett K, Ng H, Leonard JV. A combined defect of three mitochondrial carboxylases presenting as biotin-responsive 3-methylcrotonyl glycinuria and 3-hydroxyisovaleric aciduria. *Clin Chim Acta* 1980; **100**: 183.
13. Dabbagh O, Brismar J, Gascon GG, Ozand PT. The clinical spectrum of biotin-treatable encephalopathies in Saudi Arabia. *Brain Dev* 1994; **16**: 72.
14. Cowan MJ, Wara DW, Packman S *et al.* Multiple biotin-dependent carboxylase deficiencies associated with defects in T-cell and B-cell immunity. *Lancet* 1979; **2**: 115.
15. Sander JE, Malamud N, Cowan MJ *et al.* Intermittent ataxia and immunodeficiency with multiple carboxylase deficiencies: a biotin-responsive disorder. *Ann Neurol* 1980; **8**: 544.
16. Munnich A, Saudubray JM, Ogier H *et al.* Deficit multiple des carboxylases. Une maladie métabolique vitamino-dépendante curable par la biotine. *Arch Fr Pédiatr* 1981; **38**: 83.
17. Schubiger G, Caflish U, Baumgartner R *et al.* Biotinidase deficiency: clinical course and biochemical findings. *J Inherit Metab Dis* 1984; **7**: 129.
18. Salbert BA, Pellock JM, Wolf B. Characterization of seizures associated with biotinidase deficiency. *Neurology* 1993; **43**: 1351.
19. Kalayci O, Coskun T, Tokatli A *et al.* Infantile spasms as the initial symptom of biotinidase deficiency. *J Pediatr* 1994; **124**: 103.
20. Charles BM, Hosking G, Green A *et al.* Biotin-responsive alopecia and developmental regression. *Lancet* 1979; **2**: 118.
21. Keeton BR, Moosa A. Organic aciduria: Treatable cause of floppy infant syndrome. *Arch Dis Child* 1981; **51**: 636.
22. Mitchell G, Ogier H, Munnich A *et al.* Neurological deterioration and lactic acidemia in biotinidase deficiency. A treatable condition mimicking Leigh's disease. *Neuropediatrics* 1986; **17**: 129.
23. Baumgartner ER, Suormala TU, Wick H *et al.* Biotinidase deficiency: a cause of subacute necrotizing encephalomyelopathy (Leigh syndrome). Report of a case with a lethal outcome. *Pediatr Res* 1989; **26**: 260.
24. Dionisi-Vici C, Bachmann C, Graziani MC, Sabetta G. Laryngeal stridor as a leading symptom in a biotinidase-deficient patient case report. *J Inherit Metab Dis* 1988; **11**: 312.
25. Tokatli A, Coskun T, Ozalp I, Gunay M. The major presenting symptom in a biotinidase-deficient patient: laryngeal stridor. *J Inherit Metab Dis* 1992; **15**: 281.
26. DiRocco M, Superti-Furga A, Caprino D, Oddino N. Letter: Phenotypic variability in biotinidase deficiency. *J Pediatr* 1984; **104**: 964.
27. Wolf B, Grier RE, Heard GS. Hearing loss in biotinidase deficiency. *Lancet* 1983; **2**: 1365.
28. Taitz LS, Green A, Strachan I *et al.* Biotinidase deficiency and the eye and ear. *Lancet* 1983; **1**: 918.
29. Lott IT, Lottenberg S, Nyhan WL, Buchsbaum MJ. Cerebral metabolic change after treatment in biotinidase deficiency. *J Inherit Metab Dis* 1993; **16**: 399.
30. Campana G, Valentini G, Legnaioli MI *et al.* Ocular aspects of biotinidase deficiency: clinical and genetic original studies. *Ophthalm Paediatr Genet* 1987; **8**: 125.

31. Salbert BA, Astruc J, Wolf B. Ophthalmologic findings in biotinidase deficiency. *Ophthalmologica* 1993; **206**: 177.
32. Leonard JV, Daish P, Naughten ER, Bartlett K. The management and long term outcome of organic acidaemias. *J Inherit Metab Dis* 1984; **7**: 13.
33. Wolf B, Spencer R, Gleason T. Hearing loss is a common feature of symptomatic children with profound biotinidase deficiency. *J Pediatr* 2002; **140**: 242.
34. Thuy LP, Zielinska B, Zammarchi E *et al*. Multiple carboxylase deficiency due to deficiency of biotinidase. *J Neurogenet* 1986; **3**: 357.
35. Ramaekers VT, Brab M, Rau G, Heimann G. Recovery from neurologic deficits following biotin treatment in a biotinidase Km variant. *Neuropediatrics* 1993; **24**: 98.
36. Ramaekers VT, Suormala TM, Brab M *et al*. A biotinidase Km variant causing late onset bilateral optic neuropathy. *Arch Dis Child* 1992; **67**: 115.
37. Wolf B, Pomponio RJ, Norrgard KJ *et al*. Delayed-onset profound biotinidase deficiency. *J Pediatr* 1998; **132**: 362.
38. Williams ML, Packman S, Cowan MJ. Alopecia and periorificial dermatitis in biotin-responsive multiple carboxylase deficiency. *J Am Acad Derm* 1983; **9**: 97.
39. Fischer A, Munnich A, Saudubray JM *et al*. Biotin-responsive immunoregulatory dysfunction in multiple carboxylase deficiency. *J Clin Immun* 1982; **2**: 35.
40. Petrelli F, Moretti P, Campanati G. Studies on the relationships between biotin and the behaviour of B and T lymphocytes in the guinea pig. *Experientia* 1981; **37**: 1204.
41. Hurvitz H, Ginat-Israeli T, Elpeleg ON *et al*. Biotinidase deficiency associated with severe combined immunodeficiency. *Lancet* 1989; **2**: 228.
42. Fois A, Cioni M, Balestri P *et al*. Biotinidase deficiency: metabolites in CSF. *J Inherit Metab Dis* 1986; **9**: 284.
43. Collins JE, Nicholson NS, Dalton N, Leonard JV. Biotinidase deficiency: early neurological presentation. *Dev Med Child Neurol* 1994; **36**: 263.
44. Taitz LS, Leonard JV, Bartlett K. Long-term auditory and visual complications of biotinidase deficiency. *Early Hum Dev* 1985; **11**: 325.
45. Schulz PE, Seiner SP, Belmont JW, Fishman MA. Basal ganglia calcifications in a case of biotinidase deficiency. *Neurology* 1988; **38**: 1326.
46. Bousounis DP, Camfield PR, Wolf B. Reversal of brain atrophy with biotin treatment in biotinidase deficiency. *Neuropediatrics* 1993; **24**: 214.
47. Ginat-Israeli T, Hurvitz H, Klar A *et al*. Deteriorating neurological and neuroradiological course in treated biotinidase deficiency. *Neuropediatrics* 1993; **24**: 103.
48. Wolf B, Norrgard K, Pomponio R *et al*. Profound biotinidase deficiency in two asymptomatic adults. *Am J Med Genet* 1997; **73**: 5.
49. Wolf B, Grier RE, Allen RJ *et al*. Biotinidase deficiency: the enzymatic defect in late-onset multiple carboxylase deficiency. *Clin Chim Acta* 1983; **131**: 273.
50. Pomponio RJ, Hymes J, Pandya A *et al*. Prenatal diagnosis of heterozygosity for biotinidase deficiency by enzymatic and molecular analyses. *Prenat Diagn* 1998; **18**: 117.
51. Chalmers RA, Mistry J, Docherty PW, Stratton D. First trimester prenatal exclusion of biotinidase deficiency. *J Inherit Metab Dis* 1994; **17**: 751.
52. Swick HM, Kien CL. Biotin deficiency with neurologic and cutaneous manifestations but without organic aciduria. *J Pediatr* 1983; **103**: 265.
53. Wolf B, Heard GS, Jefferson LG *et al*. Clinical findings in four children with biotinidase deficiency detected through a statewide neonatal screening program. *N Engl J Med* 1985; **313**: 16.
54. Di Rocco M, Superti-Furga A, Durand P *et al*. Different organic acid patterns in urine and in cerebrospinal fluid in a patient with biotinidase deficiency. *J Inherit Metab Dis* 1984; **7**: 119.
55. Duran M, Baumgartner ER, Suormala TM *et al*. Cerebrospinal fluid organic acids in biotinidase deficiency. *J Inherit Metab Dis* 1993; **16**: 513.
56. Wolf B, Secor McVoy J. A sensitive radioassay for biotinidase activity: deficient activity in tissues of serum biotinidase-deficient individuals. *Clin Chim Acta* 1983; **135**: 275.
57. Thuy LP, Zielinska B, Sweetman L, Nyhan WL. Determination of biotinidase activity in human plasma using (14C) biocytin as substrate. *Ann NY Acad Sci* 1985; **447**: 434.
58. Wastell H, Dale G, Bartlett K. A sensitive fluorimetric rate assay for biotinidase using a new derivative of biotin biotinyl-6-aminoquinoline. *Anal Biochem* 1984; **140**: 69.
59. Heard GS, Wolf B, Jefferson KG *et al*. Newborn screening for biotinidase deficiency: results of a one-year pilot study. *J Pediatr* 1986; **108**: 40.
60. Wolf B. Worldwide survey of neonatal screening for biotinidase deficiency. *J Inherit Metab Dis* 1991; **14**: 923.
61. Hart PS, Hymes J, Wolf B. Biochemical and immunological characterization of serum biotinidase in profound biotinidase deficiency. *Am J Hum Genet* 1992; **50**: 125.
62. Funghini S, Donati MA, Pasquini E *et al*. Two new mutations in children affected by partial biotinidase deficiency ascertained by newborn screening. *J Inherit Metab Dis* 2002; **25**: 328.
63. Pomponio RJ, Coskun T, Demirkol M *et al*. Novel mutations cause biotinidase deficiency in Turkish children. *J Inherit Metab Dis* 2000; **23**: 120.
64. Thoene JG, Lemons R, Baker H. Impaired intestinal absorption of biotin in juvenile multiple carboxylase deficiency. *N Engl J Med* 1983; **308**: 639.
65. Bonjour JP, Bausch J, Suormala T, Baumgartner ER. Detection of biocytin in urine of children with congenital biotinidase deficiency. *Intl J Vitam Nutr Res* 1984; **54**: 223.
66. Suormala TM, Baumgartner ER, Bausch J *et al*. Quantitative determination of biocytin in urine of patients with biotinidase deficiency using high-performance liquid chromatography (HPLC). *Clin Chim Acta* 1988; **177**: 253.
67. Bartlett K, Ng H, Dale G *et al*. Studies on cultured fibroblasts from patient with defects of biotin-dependent carboxylation. *J Inherit Metab Dis* 1981; **4**: 183.
68. Leonard JV, Seakins JWT, Bartlett K *et al*. Inherited disorders of 3-methylcrotonyl-CoA carboxylation. *Arch Dis Child* 1981; **56**: 53.

69. Packman S, Caswell NW, Baker H. Biochemical evidence for diverse etiologies in biotin-responsive multiple carboxylase deficiency. *Biochem Gen* 1982; **20**: 17.
70. Sweetman L, Bates SP, Hull D, Nyhan WL. Propionyl CoA carboxylase deficiency in a patient with biotin-responsive 3-methylcrotonylglycinuria. *Pediatr Res* 1977; **11**: 1144.
71. Suormala T, Wick H, Bonjour JP, Baumgartner ER. Rapid differential diagnosis of carboxylase deficiencies and evaluation for biotin-responsiveness in a single blood sample. *Clin Chim Acta* 1985; **145**: 151.
72. Munnich A, Saudubray J-M, Cotissson A *et al*. Biotin-dependent multiple carboxylase deficiency presenting as a congenital lactic acidosis. *Eur J Pediatr* 1981; **137**: 203.
73. Wallace SJ. Biotinidase deficiency: presymptomatic treatment. *Arch Dis Child* 1985; **60**: 574.
74. Munnich A, Saudubray JM, Coude FX *et al*. Fatty acid-responsive alopecia in multiple carboxylase deficiency. *Lancet* 1980; **1**: 1080.

Isovaleric acidemia

Introduction	57	Treatment	61
Clinical abnormalities	58	References	61
Genetics and pathogenesis	59		

MAJOR PHENOTYPIC EXPRESSION

Episodic overwhelming illness with vomiting, ketosis, acidosis, and coma; characteristic odor; neutropenia and thrombocytopenia; isovaleric acidemia; urinary excretion of isovalerylglutamine and 3-hydroxyisovaleric acid; C5 and C5/C3 acylcarnitine profile; and deficiency of isovaleryl CoA dehydrogenase.

INTRODUCTION

Isovaleric acidemia was first described in 1966 by Tanaka and colleagues [1, 2]. It was the unusual odor that led to the recognition of the disorder as an inborn error of metabolism [1, 2]. The smell, that of typical volatile, short-chain organic acid, was so recognized by two chemists, LB Sjostrim and D Tokendall, in the original patients. It was then documented as isovaleric acid by gas chromatography. The molecular defect is in the enzyme, isovaleryl CoA dehydrogenase (Figure 7.1) [3]. The gene has been localized to chromosome 15q12-15 [4, 5]. It contains 12 exons [5]. Mutations have been reported, including missense point mutations, deletions, and mutations that result in novel

processing of this mitochondrial enzyme, such as a variant that causes an mRNA splicing error deleting exon 2 and producing a truncated protein that fails to interact properly with receptors for import into mitochondria [6–10]. The advent of expanded programs of newborn screening has uncovered individuals with potentially asymptomatic isovaleric acidemia. A single mutation C932T leading to an A282V protein was found in 47 percent of mutant alleles [10]. The urinary isovalerylglutamine excretion of these patients was lower than those of a group of patients identified because they became symptomatic, as were the C5 carnitine levels in the newborn blood spots despite the fact that enzyme activity was zero. By the time of report, up to five years of age, none had become symptomatic.

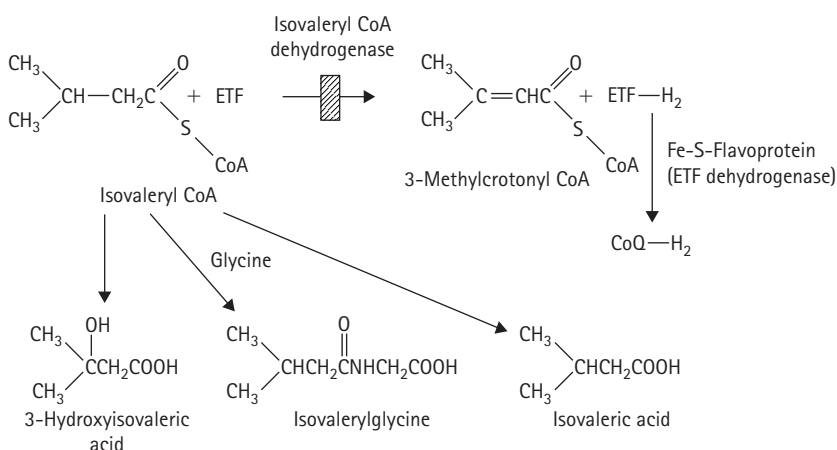


Figure 7.1 Isovaleryl CoA dehydrogenase, the site of the molecular defect in isovaleric acidemia. The characteristic urinary metabolites in this disease are isovalerylglutamine and 3-hydroxyisovaleric acid.



Figure 7.2 AAD: An infant with isovaleric acidemia in the incubator. A nasogastric tube was in place.



Figure 7.3 AAD: Close up of the face.

Furthermore, six older siblings were indentified to have the same genotype, and none of them had ever been symptomatic.

CLINICAL ABNORMALITIES

Patients with isovaleric acidemia present usually with an organic acidemia picture of acute overwhelming illness in the first days or weeks of life (Figures 7.2 and 7.3) [11–17]. The onset is usually with vomiting, but the infant may progress directly to a deep coma. Hypothermia may be present. There may be convulsions, either focal or generalized [14]. Analysis of the urine usually reveals massive ketonuria and electrolyte analysis indicates a metabolic acidosis. There may be prominent hyperammonemia early in infancy [14–17]. Hypocalcemia may be present [13]. It has been estimated that more than half of the patients die during the first episode very early in life, but the numbers of patients reported are quite small, and the proportion of patients dying undiagnosed early in life may be considerably greater. Intraventricular

hemorrhage, cerebral edema, and cerebellar hemorrhage have been described [14, 18, 19]. Vomiting may be so severe that a diagnosis of pyloric stenosis is suspected. Five infants have been treated surgically during the neonatal period, four undergoing pyloromyotomy [2, 12, 20–22]; the fifth was thought to have a duodenal band [11].

The characteristic odor of isovaleric acid may alert the physician to the diagnosis. It has been popularized as the odor of sweaty feet, but it does not smell at all like most locker rooms. It is a pungent, rather unpleasant odor. It can permeate a laboratory working with samples from an acutely ill patient. It was first recognized in the special care nursery when an isolette was opened to examine the baby. It is also important to remember that the odor may be absent at the time it would be most useful, during the acute illness of the first episode. These babies are often born in one hospital, lapse into coma, are treated with parenteral fluids to correct the acidosis or with exchange transfusion for the hyperammonemia, and then transported to a neonatal intensive care unit. By this time, the odor may be undetectable. In some patients, the odor has never been detected, even after the diagnosis was known [23] (Figure 7.4).

Patients who survive the initial episode may have recurrent attacks following infection or surgery in which there is acidosis, ketosis, and coma, much like the initial episode. Vomiting or ataxia may be an initial symptom. The odor may return. The presentation may suggest a diagnosis of Reye syndrome. Hyperglycemia may occasionally be found as it may in any infant overwhelmingly ill and this



Figure 7.4 TM: A seven-year-old girl with isovaleric acidemia. She had microcephaly. The first years of life were characterized by recurrent episodes of acidosis, dehydration, and coma.

in the presence of massive ketosis can lead to a mistaken diagnosis of diabetes mellitus [24, 25]. Treatment with insulin is potentially dangerous in such a patient.

An occasional patient has a more indolent, milder intermittent form presenting first, later in the first year, or later [23]. Episodic disease is associated with intercurrent infection or unusual intake of protein. Episodes decrease in frequency with age, probably as a consequence of decreased frequency of infection. The acute neonatal disease and the chronic, intermittent form of the disease may occur in the same family.

Pancreatitis, both acute and chronic, has been reported as a complication of isovaleric acidemia [26]. In three patients, the initial presentation was with pancreatitis. In pediatric patients with pancreatitis, investigation for organic acidemia is prudent.

Hematological abnormalities may be prominent, especially in infancy. Leukopenia and thrombocytopenia are common, and in some infants anemia and a picture of pancytopenia [11, 13–18, 27–29]. These abnormalities may be encountered during the initial or later attacks. The majority of patients surviving the initial episode are developmentally normal (Figure 7.5), but some had mild mental impairment and microcephaly [2, 13, 20, 28, 30, 31], and some severely so [29], and (Nyhan and Barshop, unpublished experience). Hypotonia is common early. Later there may be ataxia, tremor, dysmetria, extrapyramidal movements, and brisk deep tendon reflexes [29, 30]. Lesions in the basal ganglia lead to dystonia (Figures 7.5 and 7.6). One of the original patients had an unsteady gait as a teenager and had mild mental impairment, but had an uneventful pregnancy and a normal infant [32]. The electroencephalogram (EEG) may reveal slow wave



Figure 7.5 AH: A girl with isovaleric acidemia whose course has been entirely benign following the initial episode.

dysrhythmia. Among our patients, a five-year-old with truncal ataxia had mild slowing, while her neurologically normal affected sister had a normal EEG. Magnetic resonance imaging (MRI) may be normal, or may show extensive atrophy in an infant with near fatal neonatal illness and cardiac arrest [33]. Neuropathology of the infant dying in the acute episode may show cerebellar edema with herniation [14]. Spongiform changes may be seen in the white matter [11, 34–36], but less prominently than in other organic acidemias or nonketotic hyperglycinemia [36]. Histology of the liver may be that of fatty change [35]. Successful pregnancies and normal infants have been reported in women with isovaleric acidemia [32, 37].

It is now clear that a substantial number of patients identified by newborn screening may be completely asymptomatic [10]. Time will tell if this continues to hold true with further experience; the variant enzyme is thermally unstable, which could create a risk during febrile illness [38].

GENETICS AND PATHOGENESIS

Isovaleric acidemia is an autosomal recessive disease. Heterozygote detection has been carried out by assay of the conversion of leucine-2-¹⁴C to CO₂ in fibroblasts [13, 39]. Prenatal diagnosis has been approached in the same manner [40]. An accurate method for the gas chromatographic-mass spectrometric analysis of 3-hydroxyisovaleric acid [41] or isovalerylglutamate permits rapid prenatal diagnosis via direct detection in the amniotic fluid [42]. Isovalerylglutamate appears to be the metabolite of choice; it has been diagnostic as early as 12 weeks of gestation. Prenatal diagnosis has also been made by the incorporation of labeled isovaleric acid in chorionic villus material [43].

Assay of the enzyme in fibroblasts of a series of patients revealed considerable heterogeneity and residual activity, as much as 13 percent of the control level [39, 44–46]. The enzyme may also be assayed in leukocytes. The assay is not generally available. Isovaleryl CoA dehydrogenase, a member of the acylCoA dehydrogenase family, is made as a 45 kDa subunit precursor [47] and processed to a 43 kDa during import into the mitochondria and then assembled as a tetramer. It is a flavine adenine dinucleotide (FAD)-containing enzyme, whose electrons are transferred to electron transfer flavoprotein (ETF) and transmitted to coenzyme Q of the electron transport chain by ETF dehydrogenase [48].

The gene has been located on chromosome 15q13-15 spanning 12 exons over 15 kb [49]. Complementation studies of 12 patients revealed a single group, comprising acute neonatal and chronic, intermittent patients [50]. A certain number of different types of mutation has been described [8] in patients identified because of symptomatic disease. Many have been point mutations in the coding region leading to an inactive or unstable protein [8, 32, 39, 51]. A few which code for a protein with appreciable

enzyme function lead to mild clinical phenotypes [38, 52]. Of two missense mutations, one led to a leucine to proline change at position 13. These mutations lead to mature and precursor proteins of normal size. A single base deletion at position 1179 led to a frameshift and addition of eight amino acids and then a termination leading to a smaller precursor protein. A number of mutations has led to abnormal splicing of the RNA [8], including the deletion of exon 2 and the synthesis of a protein 20 amino acids smaller which was processed normally into mitochondria [6, 8]. The common missense mutation C932T discovered through newborn screening [10] has been present in homozygous and heterozygous fashion with another mutation. Restriction enzyme analysis with *Bsa*II yields a recognizable 213-bp uncleaved product, while the wild type is cleaved to a characteristic 46-bp fragment. A 15-bp insertion in intron 7 resulted from missplicing and the use of a cryptic splice acceptor site and maintenance of the correct reading frame [51].

The immediate consequence of the enzyme defect is the elevated concentration of isovaleric acid in the blood. 3-Hydroxyisovaleric acid is also prominent [53]. These elevations are especially true in the acute episode. However, methods for the detection of volatile short-chain acids like isovaleric acid are considerably less than perfect, and mistakes have been made in which the diagnosis of isovaleric acidemia was missed [54]. The way in which the diagnosis has usually been securely made is by the identification of large amounts of isovalerylglutamine in the urine [54–56]. This compound is very stable and is present in the urine even at times of remission and excellent general health. Amounts in the urine may be as great as 3 g/day (2000–9000 mmol/mol creatinine) (normal 0–2),

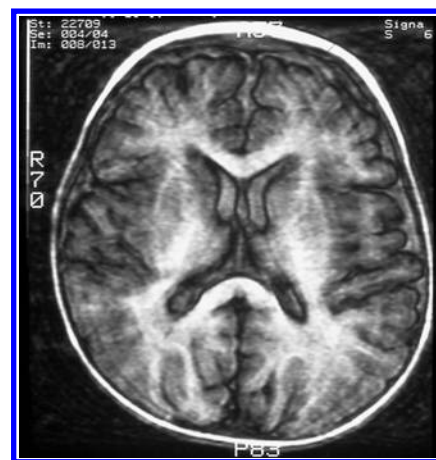


Figure 7.6 Abnormalities in the basal ganglia and white matter disease on T_2 -weighted magnetic resonance images.

whereas in normal individuals less than 2 mg is found. A simple screening test has been developed [57], but today most patients are detected by organic acid analysis.

Analysis of organic acids at the time of acute attack reveals the presence of 4-hydroxyisovaleric acid, mesaconic acid, and methylsuccinic acid [58], as well as isovalerylglutamine and 3-hydroxyisovaleric acid. Lactic acid, acetoacetic acid, and 3-hydroxybutyric acid are also found in large amounts in the urine. Isovalerylgluturonide has also been identified in the urine [59], and probably represents an additional detoxification pathway. Similarly, isovalerylcarnitine (Figure 7.7) has been identified in the urine [60], and this provides another approach to the diagnosis. After the acute attack has resolved, organic acid analysis usually

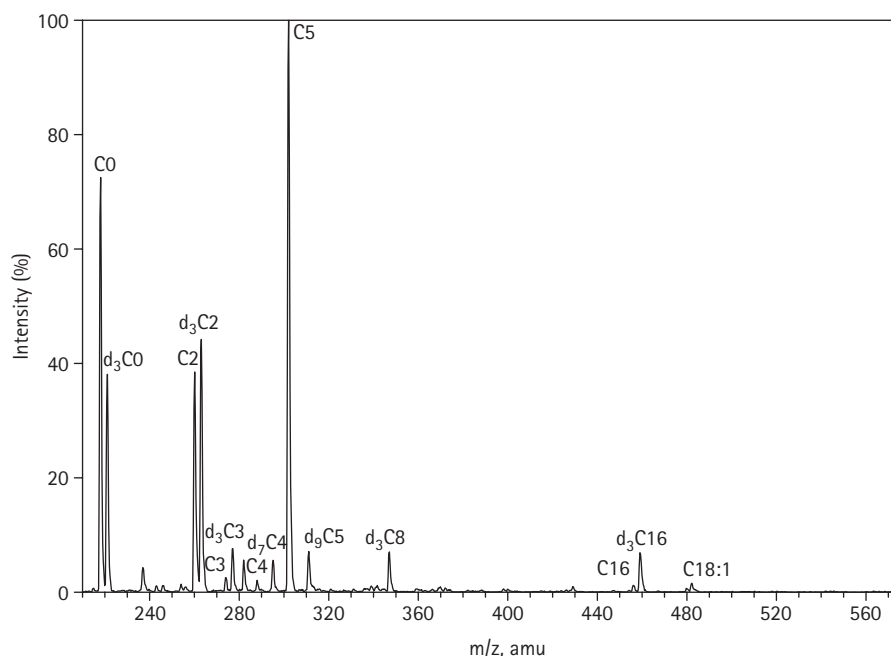


Figure 7.7 Acylcarnitine profile of the blood plasma of a patient with isovaleric acidemia. C5 is isovalerylcarnitine. (Illustration provided by Jon Gangoiti of University of California, San Diego.)

reveals only isovalerylglycine. Isovalerylglycine can also be identified by nuclear magnetic resonance (NMR) spectroscopy [61].

With the advent of tandem mass spectrometry, we can expect the diagnosis to be made increasingly by the analysis of acylcarnitine profiles either in blood spots on filter paper in programs of neonatal screening or in plasma of ill patients. An isolated elevation of C5 acylcarnitine is likely isovalerylcarnitine. 2-Methylbutyrylcarnitine is also C5; its elevation in multiple acyl CoA dehydrogenase deficiency is accompanied by C4, but isolated 2-methylbutyryl CA dehydrogenase deficiency must be distinguished by the presence of 2-methylbutyrylglycine in the urine or by enzyme assay. In isovaleric acidemia, the ratio of C5 to C3 is useful [62].

TREATMENT

The acute episode should be treated vigorously with parenteral solutions of fluid and electrolytes containing sodium bicarbonate and glucose. The initial episode, especially if complicated by hyperammonemia, may require exchange transfusions or dialysis or the use of benzoate or phenylacetate to promote waste nitrogen elimination. Intravenous carnitine may alleviate hyperammonemia and carnitine deficiency, and it may promote the excretion of isovalerylcarnitine. The administration of exogenous glycine may be helpful in the acute episode [63]. Doses employed have approximated 250 mg/kg per day.

Glycine is also employed in chronic therapy [64, 65]. It has been reported to prevent the increase in accumulation of isovaleric acid that follows an oral load of leucine [66]. A dose of 800 mg/day has been recorded for an infant [63]. In an approach to optimal use of glycine supplementation, quantification of isovalerylglycine excretion was studied [67] in two patients with disease of different severity. Different doses were employed, and one was challenged with leucine. Interestingly, the patient with the milder disease excreted much more isovalerylglycine, suggesting that disease severity may be a function of the efficiency of glycine conjugation. Doses up to 600 mg/kg appeared to be useful especially at times when isovaleric acid accumulation might be highest; doses up to 250 mg/kg might be adequate under baseline healthy conditions of dietary restriction.

Carnitine may become depleted in isovaleric acidemia. Patients tend to have low levels of free-carnitine in plasma and increased losses of esterified carnitine in urine [68–74]. Supplementation restores plasma free carnitine to normal and increases urinary excretion of isovalerylcarnitine. Studies with isotopically labeled carnitine showed that administered carnitine rapidly enters mitochondrial pools and esterifies with available acyl compounds [60]. Comparison of oral and intravenous use indicated an oral bioavailability of only 15 percent; intravenous use is required in acute episodic illness. Oral dosage of 100 mg/

kg appears adequate for chronic use. There have been conflicting results of studies to determine whether glycine or carnitine is more effective in removing isovalerylCoA [69, 72, 73]. It appears prudent to employ both in long-term management.

The cornerstone of long-term therapy is the restriction of the dietary intake of leucine [28]. Our approach to the treatment of organic acidemia is to provide whole protein containing the offending amino acid required for growth and little more. Our experience with isovaleric acidemia is that the provision of protein can be somewhat more liberal with excellent results (Figure 7.5) than in other organic acidemias such as propionic acidemia (Chapter 2) or methylmalonic acidemia (Chapter 3).

In studies of stable isotopically labeled leucine, more than 90 percent of the excreted metabolites of leucine were produced by endogenous metabolism when the whole leucine-containing protein intake was 0.75 g/kg [71]. Nutritional therapy, as well as glycine supplementation, should be monitored by quantification of the excretion of isovalerylglycine. Determinations of the concentration of amino acids in plasma ensures against any one or more amino acids reaching concentrations that would be limiting for growth. Mixtures of amino acids lacking leucine may be employed to increase amino acid nitrogen or nonleucine essential amino acids. Supplementation with alanine may accomplish a similar goal [74].

REFERENCES

1. Tanaka K, Budd MA, Efron ML, Isselbacher KJ. Isovaleric acidemia: A new genetic defect of leucine metabolism. *Proc Natl Acad Sci USA* 1966; **56**: 236.
2. Budd MA, Tanaka K, Holmes LB *et al*. Isovaleric acidemia: clinical features of a genetic defect of leucine metabolism. *N Engl J Med* 1967; **277**: 3211.
3. Rhead WR, Tanaka K. Demonstration of a specific mitochondrial isovaleryl CoA dehydrogenase deficiency in fibroblasts from patients with isovaleric acidemia. *Proc Natl Acad Sci USA* 1980; **77**: 580.
4. Tanaka K. Isovaleric acidemia: personal history clinical survey and study of the molecular basis. *Prog Clin Biol Res* 1990; **321**: 273.
5. Parimoo B, Tanaka K. Structural organization of the human isovaleryl-CoA dehydrogenase gene. *Genomics* 1993; **15**: 582.
6. Vockley J, Nagao M, Parimoo B, Tanaka K. The variant human isovaleryl-CoA dehydrogenase gene responsible for type II isovaleric acidemia determines an RNA splicing error leading to the deletion of the entire second coding exon and the production of a truncated precursor protein that interacts poorly with mitochondrial import receptors. *J Biol Chem* 1992; **267**: 2494.
7. Matsubara Y, Ito M, Glassberg R *et al*. Nucleotide sequence of messenger RNA encoding human isovaleryl coenzyme A dehydrogenase and its expression in isovaleric acidemia fibroblasts. *J Clin Invest* 1990; **85**: 1058.

8. Vockley J, Parimoo B, Tanaka K. Molecular characterization of four different classes of mutations in the isovaleryl-CoA dehydrogenase gene responsible for isovaleric acidemia. *Am J Hum Genet* 1991; **49**: 147.
9. Vockley J, Anderson BD, Willard JM *et al.* Abnormal splicing of IVD RNA in isovaleric acidemia caused by amino acid altering point mutations in the IVD gene: a novel molecular mechanism for disease. *Am J Hum Genet* 1998; **63**: A14.
10. Ensenauer R, Vockley J, Willard J-M *et al.* A common mutation is associated with a mild potentially asymptomatic phenotype in patients with isovaleric acidemia diagnosed by newborn screening. *Am J Hum Genet* 2004; **75**: 1136.
11. Newman CGH, Wilson BDR, Callaghan P, Young L. Neonatal death associated with isovaleric acidemia. *Lancet* 1967; **2**: 439.
12. Spirer Z, Swirsky-Fein S, Zakut V *et al.* Acute neonatal isovaleric acidemia: a report of two cases. *Israel J Med Sci* 1976; **11**: 1055.
13. Saudubray J-M, Sorin M, Depondt E *et al.* Acidemie isovalerique: étude et traitement chez trois frères. *Arch Franc Ped* 1976; **33**: 795.
14. Fischer AQ, Challa VR, Burton BK, McLean WT. Cerebellar hemorrhage complicating isovaleric acidemia: a case report. *Neurology* 1981; **31**: 746.
15. Wilson WG, Audenaert SM, Squillaro EJ. Hyperammonaemia in a preterm infant with isovaleric acidemia. *J Inherit Metab Dis* 1984; **7**: 71.
16. Mendiola JJ, Robotham JL, Liehr JG, Williams JC. Neonatal lethargy due to isovaleric acidemia and hyperammonemia. *Tex Med J* 1984; **80**: 52.
17. Beauvais P, Peter MO, Barbier B. [Neonatal form of isovaleric acidemia: Apropos of a new case.] *Arch Franc Pediatr* 1985; **42**: 531.
18. Berry GT, Yudkoff M, Segal S. Isovaleric acidemia: medical and neurodevelopmental effects of long-term therapy. *J Pediatr* 1988; **113**: 58.
19. Truscott RJW, Malegan D, McCairns E *et al.* New metabolites in isovaleric acidemia. *Clin Chim Acta* 1981; **110**: 187.
20. Lehnert W, Schenck W, Niederhof H. Isovaleric acidemia kombiniert mit hypertrophischer Pylorusstenose. *Klin Paediat* 1979; **191**: 477.
21. Nyhan WL. Introduction. In: Nyhan WL (ed.). *Abnormalities in Amino Acid Metabolism in Clinical Medicine*. Norwalk, CT: Appleton Century Crofts, 1984: 3–18.
22. Ichiba Y, Sato K, Yuasa S. Report of a case of isovaleric acidemia. *J Japan Pediatr Soc* 1979; **83**: 480.
23. Ando T, Klingberg WD, Ward AN *et al.* Isovaleric acidemia presenting with altered metabolism of glycine. *Pediatr Res* 1971; **5**: 478.
24. Williams KM, Peden VH, Hillman RE. Isovaleric acidemia appearing as diabetic ketoacidosis. *Am J Dis Child* 1981; **135**: 1068.
25. Attia N, Sakati N, Al Ashwal A *et al.* Isovaleric acidemia appearing as diabetic ketoacidosis. *J Inherit Metab Dis* 1996; **19**: 85.
26. Kahler SG, Sherwood WG, Woolf D *et al.* Pancreatitis in patients with organic acidemias. *Pediatrics* 1994; **124**: 239.
27. Kelleher J, Yudkoff M, Hutchinson R *et al.* The pancytopenia of isovaleric acidemia. *Pediatrics* 1980; **65**: 1023.
28. Levy HL, Erickson A, Lott IT, Kurtz DJ. Isovaleric acidemia. Results of family study and dietary treatment. *Pediatrics* 1973; **52**: 83.
29. Guibaud P, Divry P, Dubois Y *et al.* Une observation d'acidemie isovalerique. *Arch Franc Ped* 1973; **30**: 633.
30. Rousson R, Guibaud P. Long term outcome of organic acidurias: survey of 105 French cases (1967–1983). *J Inherit Metab Dis* 1984; **7**: 10.
31. Dodelson de Kremer R, Depetris de Boldini C, Paschini de Capra A *et al.* Variacion en la expresion fenotopica de la acidemia isovalerica en pacientes argentinos. Observaciones de un prolongado seguimiento. *Medicina* 1992; **52**: 131.
32. Krieger I, Tanaka K. Therapeutic effects of glycine in isovaleric acidemia. *Pediatr Res* 1976; **10**: 25.
33. Shih VE, Aubry RH, DeGrande G *et al.* Maternal isovaleric acidemia. *J Pediatr* 1984; **105**: 77.
34. Malan C, Neethling AC, Shanley BC *et al.* Isovaleric acidemia in two South African children. *S Afr Med J* 1977; **51**: 980.
35. Spirer Z, Swirsky-Fein S, Zakut V *et al.* Acute neonatal isovaleric acidemia: a report of two cases. *Israel J Med Sci* 1975; **11**: 1005.
36. Shuman RM, Leech RW, Scott CR. The neuropathology of the nonketotic and ketotic hyperglycinemias: three cases. *Neurology* 1978; **28**: 139.
37. Vockley J, Ensenauer R. Isovaleric acidemia: New aspects of genetic and phenotypic heterogeneity. *Am J Med Genet C Semin Med Genet* 2006; **142**: 95.
38. Nasser I, Mohsen AW, Jelesarov I *et al.* Thermal unfolding of medium chain acylCoA dehydrogenase and iso(3)valeryl-CoA dehydrogenase: study of the effect of genetic defects on enzyme stability. *Biochim Biophys Acta* 2004; **1690**: 22.
39. Shih VE, Mandell R, Tanaka K. Diagnosis of isovaleric acidemia in cultured fibroblasts. *Clin Chim Acta* 1973; **48**: 437.
40. Blaskovics ME, Ng WG, Donnell GN. Prenatal diagnosis and a case report of isovaleric acidemia. *J Inherit Metab Dis* 1978; **1**: 9.
41. Jakobs C, Sweetman L, Nyhan WL, Packman S. Stable isotope dilution analysis of 3-hydroxyisovaleric acid in amniotic fluid: contribution to the prenatal diagnosis of inherited disorders of leucine catabolism. *J Inherit Metab Dis* 1984; **7**: 15.
42. Hine DG, Hack AM, Goodman SI, Tanaka K. Stable isotope dilution analysis of isovaleryl-glycine in amniotic fluid and urine and its application for the prenatal diagnosis of isovaleric acidemia. *Pediatr Res* 1986; **20**: 222.
43. Kleijer WJ, Van Der Kraan M, Huijman JGM *et al.* Prenatal diagnosis of isovaleric acidemia by enzyme and metabolite assay in the first and second trimesters. *Prenat Diagn* 1995; **15**: 527.
44. Rhead WJ, Hall CL, Tanaka K. Novel tritium release assays for isovaleryl CoA dehydrogenases. *J Biol Chem* 1981; **256**: 1616.
45. Hyman DB, Tanaka K. Isovaleryl-CoA dehydrogenase activity in isovaleric acidemia fibroblasts using an improved tritium release assay. *Pediatr Res* 1986; **20**: 59.
46. Frerman FE, Goodman SI. Fluorometric assay of acyl CoA dehydrogenases in normal and mutant fibroblasts. *Biochem Med* 1985; **33**: 38.

47. Ikeda Y, Fenton WA, Tanaka K. *In vitro* translation and posttranslational processing of four mitochondrial acyl-CoA dehydrogenases. *Fed Proc* 1984; **43**: 2024.
48. Ikeda Y, Keese SM, Fenton WA, Tanaka K. Biosynthesis of four rat liver mitochondrial acyl-CoA dehydrogenases: *in vitro* synthesis import into mitochondria and processing of their precursors in a cell-free system and in cultured cells. *Arch Biochem Biophys* 1987; **252**: 662.
49. Kraus JP, Matsubara Y, Barton D *et al.* Isolation of cDNA clones coding for rat isovaleryl-CoA dehydrogenase and assignment of the gene to human chromosome 15. *Genomics* 1987; **1**: 264.
50. Dubiel B, Dabrowski C, Wetts R, Tanaka K. Complementation studies of isovaleric acidemia and glutaric aciduria type II using cultured skin fibroblasts. *J Clin Invest* 1983; **72**: 1543.
51. Vockley J, Rogan PK, Anderson BD *et al.* Exon skipping in IVD RNA processing in isovaleric acidemia caused by point mutations in the coding region of the IVD gene. *Am J Hum Genet* 2000; **66**: 356.
52. Mohsen AW, Vockley J. Identification of the active site catalytic residue in human isovaleryl-CoA dehydrogenase. *Biochemistry* 1995; **34**: 10146.
53. Tanaka K, Orr JC, Isselbacher KJ. Identification of 3-hydroxyisovaleric acid in the urine of a patient with isovaleric acidemia. *Biochim Biophys Acta* 1968; **152**: 638.
54. Ando T, Nyhan W, Bachmann C *et al.* Isovaleric acidemia: Identification of isovalerate isovalerylglycine and 3-hydroxyisovalerate in urine of a patient previously reported as having butyric and hexanoic acidemia. *J Pediatr* 1973; **82**: 243.
55. Tanaka K, Isselbacher KJ. The isolation and identification of N-isovalerylglycine from urine of patients with isovaleric acidemia. *J Biol Chem* 1967; **242**: 2966.
56. Tanaka K, West-Dull A, Hine DG *et al.* Gas-chromatographic method of analysis for urinary organic acids. II: Description of the procedure and its application to diagnosis of patients with organic acidurias. *Clin Chem* 1980; **26**: 1847.
57. Ando T, Nyhan WL. A simple screening method for detecting isovaleric acidemia. *Clin Chem* 1970; **16**: 420.
58. Lehnert W, Niederhof H. 4-Hydroxyisovaleric acid: a new metabolite in isovaleric acidemia. *Eur J Pediatr* 1981; **136**: 281.
59. Hine DG, Tanaka K. The identification and the excretion pattern of isovaleryl glucuronide in the urine of patients with isovaleric acidemia. *Pediatr Res* 1984; **18**: 508.
60. Van Hove JL, Kahler SG, Millington DS *et al.* Intravenous L-carnitine and acetyl-L-carnitine in medium-chain acyl-coenzyme A dehydrogenase deficiency and isovaleric acidemia. *Pediatr Res* 1994; **35**: 96.
61. Lehnert W, Hunkler D. Possibilities of selective screening for inborn errors of metabolism using high-resolution ¹H-FT-NMR spectrometry. *Eur J Pediatr* 1986; **145**: 260.
62. Vreken P, van Lint AEM, Bootsma AH *et al.* Rapid diagnosis of organic acidemias and fatty acid oxidation defects by quantitative electrospray tandem-MS acyl-carnitine analysis in plasma. In: Quant PA, Eaton S (eds). *Current Views of Fatty Acid Oxidation and Ketogenesis: From Organelles to Point Mutations*. New York: Kluwer Academic/Plenum Publishers, 1999: 327–37.
63. Cohn RM, Yudkoff M, Rothman R, Segal S. Isovaleric acidemia: use of glycine therapy in neonates. *N Engl J Med* 1978; **299**: 966.
64. Yudkoff M, Cohn RM, Puschak R *et al.* Glycine therapy in isovaleric acidemia. *J Pediatr* 1978; **92**: 813.
65. Levy HL, Erickson AM. Isovaleric acidemia. In: Nyhan WL (ed.). *Heritable Disorders of Amino Acid Metabolism*. New York: John Wiley & Sons, 1974: 81–97.
66. Naglak M, Salvo R, Madsen K *et al.* The treatment of isovaleric acidemia with glycine supplement. *Pediatr Res* 1988; **24**: 9.
67. Roe CR, Millington DS, Maltby DA *et al.* L-carnitine therapy in isovaleric acidemia. *J Clin Invest* 1984; **74**: 2290.
68. De Sousa C, Chalmers RA, Stacey TE *et al.* The response to L-carnitine and glycine therapy in isovaleric acidemia. *Eur J Pediatr* 1986; **144**: 451.
69. Chalmers RA, Roe CR, Stacey TE, Hoppel CL. Urinary excretion of L-carnitine and acylcarnitines by patients with disorders of organic acid metabolism: evidence of secondary insufficiency of L-carnitine. *Pediatr Res* 1984; **18**: 1325.
70. Stanley CA, Hale DE, Whiteman DEH *et al.* Systemic carnitine (carn) deficiency in isovaleric acidemia. *Pediatr Res* 1983; **17**: 296 a.
71. Fries MH, Rinaldo P, Schmidt-Sommerfeld E *et al.* Isovaleric acidemia: response to a leucine load after three weeks of supplementation with glycine L-carnitine and combined glycine-carnitine therapy. *J Pediatr* 1998; **129**: 449.
72. Itoh T, Ito T, Ohba S *et al.* Effect of carnitine administration on glycine metabolism in patients with isovaleric acidemia: significance of acetylcarnitine determination to estimate the proper carnitine dose. *Tohoku J Exp Med* 1996; **179**: 101.
73. Millington DS, Roe CR, Maltby DA, Inoue F. Endogenous catabolism is the major source of toxic metabolites in isovaleric acidemia. *J Pediatr* 1987; **110**: 56.
74. Wolff JA, Kelts DG, Algert S *et al.* Alanine decreases the protein requirements of infants with inborn errors of amino acid metabolism. *J Neurogenet* 1985; **2**: 41.

Glutaric aciduria (type I)

Introduction	64	Treatment	71
Clinical abnormalities	64	References	72
Genetics and pathogenesis	69		

MAJOR PHENOTYPIC EXPRESSION

Megalencephaly; acute encephalitis-like crises; neurodegenerative disorder with spasticity, dystonia, choreoathetosis, ataxia, and dyskinesia; seizures, increased signal on imaging of caudate and putamen and characteristic frontotemporal atrophy; glutaric aciduria and 3-hydroxyglutaric aciduria; and deficient activity of glutaryl CoA dehydrogenase.

INTRODUCTION

Glutaric aciduria was first described by Goodman *et al.* [1] in two siblings who began at three and seven months of age to have a neurodegenerative disorder characterized by opisthotonos, dystonia, and spasticity. One had a chronic compensated metabolic acidosis in which the serum bicarbonate concentration ranged from 7.5 to 15.7 mEq/L. It has now become apparent that macrocephaly is a prominent, often the initial, manifestation in infancy [2, 3].

The cause of this disease is deficiency in the activity of glutaryl CoA dehydrogenase. This enzyme is on the pathway for the catabolism of lysine, hydroxylysine, and tryptophan (Figure 8.1). This pathway is also the site of the defect in 2-oxoadipic aciduria.

The disorder provides an argument for organic acid analysis in patients with dystonic cerebral palsy [4] and with megalencephaly. Diagnostic difficulty in infancy is highlighted by the fact that glutaric aciduria may be absent, even at times of acute neurologic decompensation [3, 5]. Some patients are identified by the presence of 3-hydroxyglutaric acid rather than glutaric acid in the urine [6, 7]. Analysis of organic acids in the cerebrospinal fluid [5, 8] or enzyme assay may be required for diagnosis. The presence of glutarylcarnitine in blood or urine may also be diagnostic, and the assay of blood spots forms the basis for neonatal screening [9, 10].

Glutaryl CoA dehydrogenase has been mapped to chromosome 19p13.2 [11]. The gene contains 11 exons over 7 kb [12]. Nearly 100 mutations have been identified

and most patients are heterozygous for two different mutations [13]. Mutations common in inbred populations are 1VS1+56>T mutation in Indians in Island Lake, Canada [14] and A421V in the Old Order Amish in Lancaster County, Pennsylvania [12].

CLINICAL ABNORMALITIES

Megalencephaly may be present at birth [3] and may necessitate cesarean section, or it may develop in the first weeks or months of life [2, 3]. By six months, head circumference may be well above the 98th percentile [2] or 2–5 SD above the mean [3]. At this time, magnetic resonance imaging (MRI) or computed tomography (CT) may reveal only increased signal intensity in the white matter, especially in the putamen and caudate. Some patients may have frontotemporal atrophy early. The neuroradiological studies are usually ordered to rule out hydrocephalus, and they do. Macrocephaly is not found in every patient, but in a series of 11 infants [3] it was present in all but two, and these two never had an acute encephalopathic crisis. A real clue to early diagnosis is the crossing of percentiles for head growth; this acceleration is maximal at three to nine months.

Patients with or without macrocephaly develop normally until the initial neurologic presentation, which may be at 2 to 37 months. The mean age of onset of the encephalopathic episode is 14 months. However, it is now clear that many of these infants considered

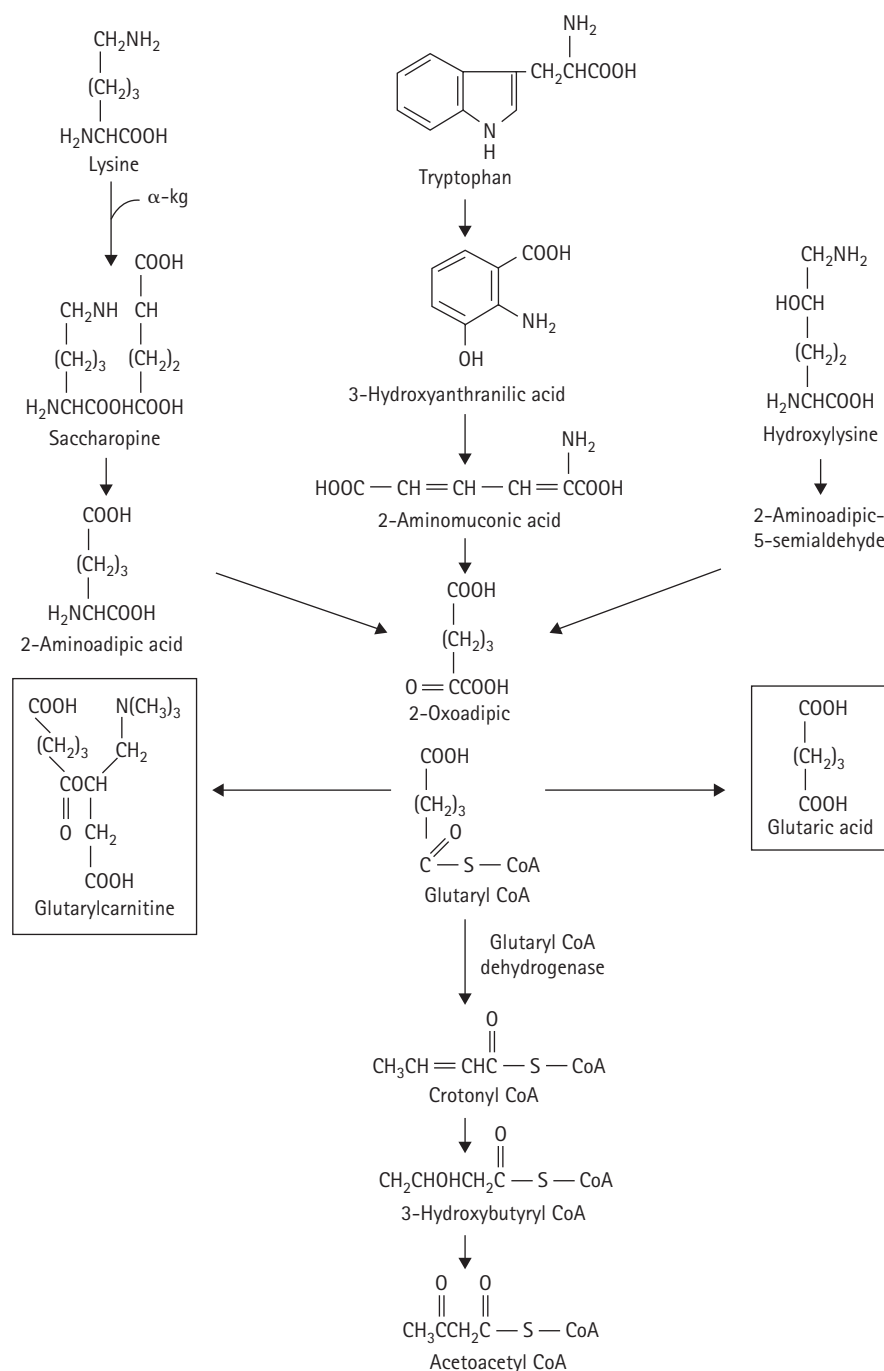


Figure 8.1 Catabolic pathways for lysine and tryptophan and the site of the defect in glutaryl CoA dehydrogenase.

to be presymptomatic may have hypotonia, jitteriness, irritability, or vomiting. Most present with an acute encephalopathic episode, often preceded by an infection and often accompanied by fever, so that an initial diagnosis of encephalitis is commonly made. The episode is characterized by acute loss of functions, such as head control, sucking and swallowing reflexes, and the ability to sit, pull to standing, or grasp toys [15]. Examination reveals profound hypotonia. There may be dystonic or athetoid movements and stiffness. There may be convulsions and paroxysmal abnormalities of the EEG. An increase in cerebrospinal fluid concentration of protein may further

suggest a diagnosis of encephalitis [16]. Recovery from the acute episode is slow and incomplete, leaving evidence of developmental deficiency and dystonia or dyskinesia (Figures 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, and 8.9) [17–23]. There may be hypotonia, grimacing, opisthotonos, rigidity, clenched fists, or tongue-thrusting. There may be repeated episodes associated with catabolic situations, in which each is followed by further evidence of neurologic deterioration. Cognitive function may initially be spared, but progressive impairment may occur. Some patients do not have acute episodes; instead, the course is one of slow neurologic degeneration. The ultimate picture of spastic,



Figure 8.2 SS: An eight-month-old infant with glutaric aciduria. She was macrocephalic from birth, but seemed otherwise well prior to encephalopathic crisis. Illustration kindly provided by Dr Georg Hoffmann of the University of Heidelberg. A black and white version was published in *Pediatrics* [3].



Figure 8.4 JS: An unrelated patient with glutaric aciduria who had been macrocephalic at birth. She had not yet had an encephalopathic crisis by two years of age. However, by five years of age after an encephalopathic crisis, she was neurologically devastated. Illustration kindly by Dr Georg Hoffmann of the University of Heidelberg.



Figure 8.3 SS: At 14 months, after encephalopathic crisis. Illustration kindly provided by Dr Georg Hoffmann of the University of Heidelberg. A black and white version was published in *Pediatrics* [3].



Figure 8.5 AS: An 18-month-old boy with glutaric aciduria illustrating the dystonic posturing and facial grimacing. He had developed normally until an initial episode at seven months during which the cerebrospinal fluid (CSF) protein was 500 mg/dL.

dystonic cerebral palsy and mental deficiency is the same. On the other hand, the course may be quite variable, even among siblings; for instance, one sib at four years could not sit, while his eight-year-old brother was doing well in school [17]. At the other extreme, two asymptomatic homozygous individuals have been observed [18, 24], but these patients also had neuroradiographic evidence of frontotemporal atrophy. We have studied [6] siblings with pronounced dystonia who were intellectually normal and had normal MRI scans of the brain.

Profuse sweating is another common manifestation [3]. Some patients have had repeated episodes of unexplained fever (hyperpyrexemic crises), irritability, ill temper, anorexia, and insomnia. Some have had hepatomegaly. Death in a Reye-like syndrome has been reported [19]. Death often occurs before the end of the first decade [17, 20–22].

Episodes of metabolic imbalance, ketoacidosis, or hypoglycemia that characterize most organic acidurias do not generally occur in this disorder. Low levels of bicarbonate may be seen chronically [1] or during acute episodes of illness [3], but in some patients they have always been normal [21, 22]. Rarely there may be ketosis, hypoglycemia, hyperammonemia and elevated levels of



Figure 8.6 AT: A 15-month-old boy with glutaric aciduria. He was dystonic and the legs scissored.



Figure 8.7 AM: This 20-month-old girl had spastic quadriplegia and opisthotonic posturing of the head.

transaminases in the blood during the acute episodes [16, 18–25].

An unusual clinical occurrence is rhabdomyolysis, as seen in disorders of fatty acid oxidation [26]. The patient reported had three episodes, the last one fatal. Levels of creatine kinase ranged from 78,000 to 189,000 IU.

Neuroradiographic findings are characteristically frontotemporal atrophy on CT or MRI with increased CSF-containing spaces in the sylvian fissures and anterior to the temporal lobes (Figures 8.10, 8.11, 8.12, 8.13, 8.14 and 8.15) [3, 18, 25, 27–30]. This may be manifest *in utero* or may occur in infancy antedating neurologic symptoms [3]; or it may develop after the first encephalopathic episode. In these latter patients, the initial abnormal finding may be



Figure 8.8 FQOM: A 15-month-old boy with glutaric aciduria. Dystonic posturing was associated with athetoid movements of the hands.



Figure 8.9 A 47-year-old Saudi man with glutaric aciduria. He came to attention because of a cousin with the disease and severe dystonia, chorea, and opisthotonus. This man, her uncle, had some dystonia of the hands on intention or excitement and imperfect gait. He had glutaric aciduria and the classic tandem mass spectrometry findings. The mutation found by Dr S Goodman of the University of Colorado: a leucine 179 arginine which has not been reported in another family. Two children in this family were diagnosed as having the disease, but have had no neurological abnormalities.

decreased attenuation in the cerebral white matter on CT or increased signal intensity on MRI of the basal ganglia [2], but enlargement of the sylvian fissure may be seen prior to the changes in the basal ganglia [25]. Reduced density in the caudate has been found by ultrasound [3]. Subdural



Figure 8.10 A nine-year-old girl with glutaryl CoA dehydrogenase who excreted 3-hydroxyglutaric acid [6]. Photographed walking, she illustrated a wide-based, dystonic gait.

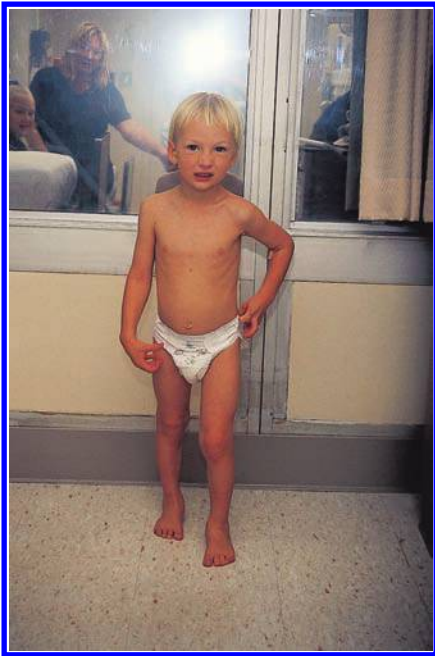


Figure 8.11 The three-year-old brother of the girl shown in Figure 8.10 displayed dystonic grimacing, athetoid posturing of the arms and hands, and a somewhat broad gait.

collections of fluid have been observed in a number of patients (Figure 8.13) [25, 30]. There may be hygromas or actual subdural hematomas [31–33], because of rupture of bridging veins stretched by the enlargement of these spaces. These occurrences have given rise to a suspicion of child

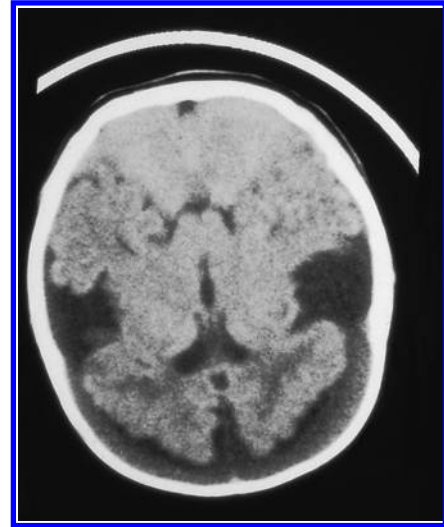


Figure 8.12 Computed tomography scan of the brain of a 13-month-old infant with glutaric aciduria. The patient was presymptomatic, but there was extensive frontotemporal atrophy. Illustration kindly provided by Dr Georg Hoffmann of the University of Heidelberg.

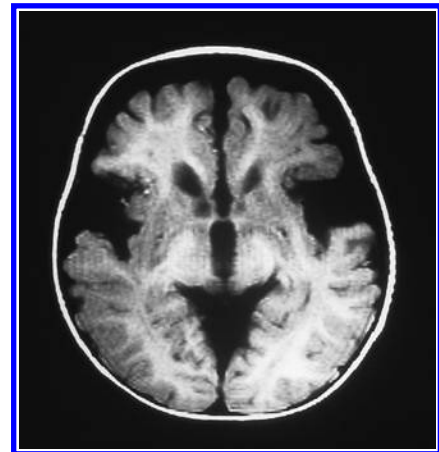


Figure 8.13 Magnetic resonance image of the patient in Figure 8.5, illustrating extreme loss of volume and the pattern of frontotemporal atrophy. Illustration kindly provided by Dr Georg Hoffmann of the University of Heidelberg.

abuse. Retinal hemorrhages may add to this suspicion. Certainly, this disease is not the most common cause of this syndrome resembling nonaccidental trauma, but it is reasonable to be sure to exclude glutaric aciduria in any such patient without other obvious signs of trauma.

The neuropathology [28, 34, 35] is that of extensive striatal neurotoxicity. There is neuronal loss and astrocytic proliferation in the caudate nucleus and the putamen, and, in some, in the globus pallidus. Changes tend to be more extensive in older patients [35]. Prominent spongiform change is seen predominantly in the white matter. Despite the cortical atrophy reported on imaging studies,

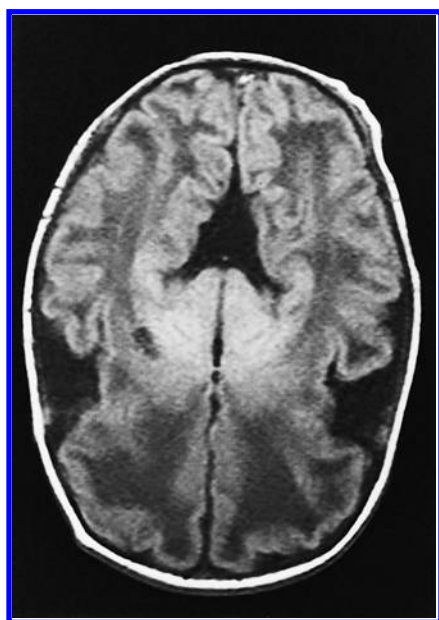


Figure 8.14 Computed tomography scan of the brain of a 3-week-old with glutaric aciduria, illustrating the early occurrence of frontotemporal atrophy. Illustration kindly provided by Dr Georg Hoffmann of the University of Heidelberg.

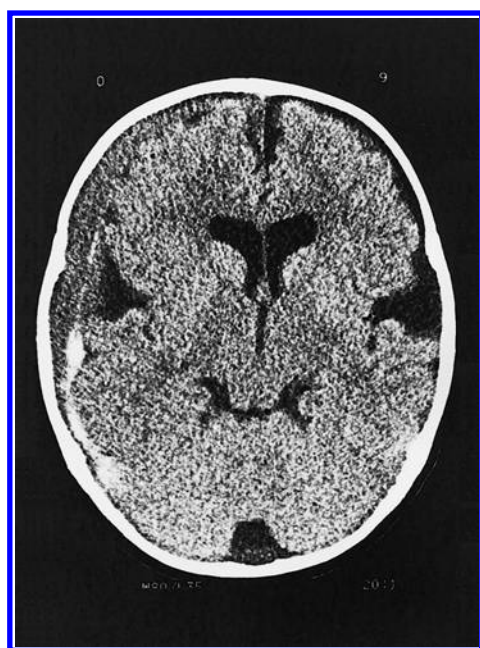


Figure 8.15 Computed tomography scan of the same patient at nine months, illustrating the development of a chronic subdural hematoma. Illustration kindly provided by Dr Georg Hoffmann of the University of Heidelberg.

neuronal loss was not found in the cortex. There may be microvesicular lipid in the liver.

The natural history and outcome in this disease has been assessed by an international group [36] that assembled

data on 279 patients, 185 of whom were diagnosed after a clinical presentation and 61 of whom were diagnosed presymptomatically – 23 by newborn screening, 24 by high risk screening, and 14 because of macrocephaly. Some highlights included the fact that the first crisis usually occurred in infancy; actually 95 percent by the age of two years, the median age was nine months. The oldest age at which a repeat crisis occurred was 70 months, indicating the importance of focusing on their prevention during the six years of life. These data were similar to the personal experience of Strauss and colleagues [37] with 77 personally observed patients over 14 years, 78 percent of whom were diagnosed after the development of striatal necrosis, the onset of which was between two and 18 months; no child in this series developed basal ganglia injury after the second birthday.

Among 49 children in the International Cross-sectional Study who died, the median age was 79 months (range, 5–490 months). The 50 percent estimated survival time was 25 years; death was most commonly as a result of aspiration pneumonia. In general, the younger the age of onset at first crisis, the more likely was a patient to die. This conclusion was similar to that arrived at in review of 115 published patients [38] in which age of onset, and not any other variable, predicted outcome, at least in those without a precipitating illness. Also, neuroimaging evidence (basal ganglia degeneration and enlargement of CSF-containing spaces) were indicative of a poor outcome.

GENETICS AND PATHOGENESIS

The site of the molecular defect is in glutaryl CoA dehydrogenase (Figure 8.1). Activity is most commonly measured in fibroblast or leukocyte lysates in which residual activity is virtually undetectable [18, 39, 40]. The disease is transmitted as an autosomal recessive trait. Intermediate activities of the enzyme have been documented in leukocytes and fibroblasts of heterozygotes [18], and consanguinity has been observed [16, 17].

The enzyme (EC 1.3.99.7) is a flavoprotein mitochondrial dehydrogenase. Its electrons are transferred to ubiquinone in interactions with the electron transfer flavoprotein (ETF) and its dehydrogenase (ETF: ubiquinone oxidoreductase). FAD is bound to the enzyme. Paper chemistry would indicate that glutaconyl CoA was an intermediate in the reaction, but if so it must remain bound to the enzyme because the only products of labeled substrate are crotonyl CoA and CO_2 [41, 42].

The cDNA for the enzyme has been cloned and sequenced. There are 11 exons. The gene has been mapped to chromosome 19p13.2 [11]. The mutation (IV1+5gtot) that has been found in homozygous Indians in Manitoba, in a population in which glutaric aciduria is common, is a splicing mutation [43, 44]. The G to T transversion in intron 1 at position +5 changes a donor splice site to Ggtcatt, which permits variable splicing, some normal but most

using a cryptic donor site 26 bases upstream and leading to a deletion of 26 base pairs, removing eight amino acids and causing a translational frame shift. Variable amounts of normal and truncated mRNA in varying individuals would determine variable phenotypes. In this population, excretion of the key metabolites is so low that patients cannot be reliably diagnosed by gas chromatography-mass spectrometry (GCMS) or tandem mass spectrometry (MS/MS). In the Amish population, in which glutaric aciduria is also common, a C to T change at 1298 changes the alanine at 421 to valine [12].

The most common mutation in the broader population was C1240 to T, which changed arginine at 402 to tryptophan (R402W). This mutation was found in 30 percent of alleles in Spain and in 40 percent of those in Germans [45, 46]. Expression of various mutations in *E. coli* led to enzyme activities ranging from less than 17 percent of normal activity to 20 percent in the Amish mutation.

Correlations between genotype and clinical severity have been elusive, possibly because neurologic impairment is related to the occurrence of encephalopathic crises rather than any other clinical, biochemical, or molecular feature. However, certain mutations have correlated well with high excretion of glutaric and 3-hydroxyglutaric acids, and others have been found in patients with low excretion [45]. In the former group, the most frequent mutations were R402W and A293T resulting from a G913A change in exon 8. These mutations were found in the low excretor group only in heterozygosity, especially in combination with R227P or V400M which together accounted for over half of the mutant alleles in the low excretor group [45, 47]. Among eight families identified in Israel, six were of Moslem origin, and two non-Ashkenazi Jews, and eight previously unidentified mutations were found including a 1-bp deletion at 1173 [48]. The siblings with 3-hydroxyglutaric aciduria [6] were compounds of R227P and E365K.

Defective enzyme activity leads to glutaric aciduria, the feature by which the diagnosis is usually made. The amounts reported may be massive: 850–1700 mmol/mol creatinine [1] and 900–1200 mmol/mol creatinine [3]. Normal levels of glutaric acid in urine range from 0.6 to 4 mmol/mol creatinine. However, patients with smaller amounts (80–200 mmol/mol creatinine) [3] have been observed, and many patients have been reported in whom glutaric acid and other characteristic metabolites were not found in the urine [4]. Metabolites in the urine have also been observed intermittently [49]. The other characteristic metabolites found in the urine are 3-hydroxyglutaric and glutaconic acids [16]; amounts are usually less than those of glutaric acid. On the other hand, we have seen children with documented deficiency of the enzyme in whom only 3-hydroxyglutaric was found in the urine, in the absence of accumulation of glutaric acid [6]. Excretion of glutaconic acid may exceed that of 3-hydroxyglutaric acid only in an acute ketotic episode when the urine also contains

3-hydroxybutyric, acetoacetic, adipic, suberic, and sebacic acids [20].

Levels of glutaric acid in plasma have ranged from 3 to 60 $\mu\text{mol/L}$ [3], but normal levels have also been recorded. Glutaric acid is undetectable in normal plasma or cerebrospinal fluid. In patients, levels of glutaric acid in the CSF have ranged from 20 to 40 $\mu\text{mol/L}$ [3, 5]. The CSF may be the only fluid in which elevated levels are found [5]. Glutaric acid concentrations have been found to be elevated in all tissues examined [29].

Measurement of bound glutaric acid by organic acid analysis following mild alkaline hydrolysis may indicate the diagnosis in patients with normal urinary glutaric acid [50]. This is probably a reflection of the excretion of glutarylcarnitine, which may be detected by tandem mass spectrometry.

The analysis of glutarylcarnitine in blood spots has been incorporated into most programs of expanded neonatal screening [10]. There are cautions about the possibility of false negatives. A reported infant with glutaric aciduria was missed in a neonatal screening program [9]. Actually, there was glutarylcarnitine on the initial spot, but a repeat was normal, and the patient was only identified after developing dystonia at 11 months during an intercurrent infection. That state has since increased the sensitivity of the screen for this disease by adjusting the signal ratio cutoff, and now recommends a complete work up for any positive rather than a repeat screening analysis of a blood spot. With time, amounts of acylcarnitines may decrease as carnitine stores are depleted. In addition, patients with mutations which put them in the low excretor group [43, 45] have been tested for glutarylcarnitine in blood spots and gave negative results even in the presence of carnitine supplementation.

Diagnostic confusion is symbolized by the fact that classic patients may excrete no elevated glutaric acid at all, and 3-hydroxyglutaric excretion may be normal. On the other side of the coin, elevated excretion of glutaric acid (100–150 mmol/mol creatinine) has been reported [51] in a patient found not to have glutaryl CoA dehydrogenase deficiency; antibiotic treatment abolished the glutaric aciduria; so the source must have been intestinal bacteria. An additional confounder is ketosis, which has been reported [52] to cause significant increases in the excretion of 3-hydroxyglutaric acid in the urine of patients who did not have glutaryl CoA dehydrogenase deficiency. In two patients with glutaryl CoA dehydrogenase deficiency and no elevation of glutarylcarnitine in the blood, there was a sizeable excretion of glutarylcarnitine in the urine [53]. There is sufficient diagnostic uncertainty that enzyme analysis or the identification of a mutant gene is an essential criterion for diagnosis.

Most patients have low concentrations of free-carnitine in plasma and elevated levels of esterified carnitine, especially in urine [3, 18, 50]. Low muscle carnitine has been reported [18], even in an asymptomatic patient.

The incidence of the disorder has not been known,

although it has been estimated at one in 30,000 in Sweden [54] and more recent experience with newborn screening in North America, Australia, and Germany gave an incidence of one in 106,900 [55]. Increased frequency of the disease has been observed in Ojibway Indians in Manitoba [44] and in the Amish of Lancaster County, Pennsylvania [56]. In Manitoba Indians, DNA-based screening gave an incidence of one in 300 [55]. Carrier detection has been improved by assay of the enzyme in cultured interleukin-2-dependent leukocytes [57], but there was still some overlap between controls and obligate heterozygotes. Molecular analysis for mutation is the most reliable method of carrier detection. Prenatal diagnosis has been made by the detection of increased amounts of glutaric acid [58] in amniotic fluid, as well as by assay of the enzyme in cultured amniocytes. Molecular analysis for mutation is the most reliable method of prenatal diagnosis. The value of prenatal diagnosis has been questioned [18] on the basis of the existence of asymptomatic homozygotes, but these individuals had frontotemporal atrophy, and studies of intellectual function were not reported.

Considerable attention has been devoted to pathogenesis and the extraordinary vulnerability of the striatum, particularly the caudate and putamen. It has seemed likely that the accumulation of metabolites and something about the catabolic response to acute infection are relevant to neuronal damage. The similarity of structures of glutaric and glutamic acids, and the fact that glutaric and 3-hydroxyglutaric acids inhibit glutamate decarboxylase of brain [59] has led to an excitotoxic theory of neuronal damage in this disease. In striatal slice cultures, 3-hydroxyglutaric acid induced neuronal degeneration by activation of NMDA receptors [60]. Convulsions and striatal neuronal damage were caused in rats by direct striatal injection of 3-hydroxyglutaric acid [61].

TREATMENT

Treatment with carnitine and the prompt, vigorous intervention in intercurrent illness with the provision of energy from glucose, water, and electrolytes appears increasingly likely to prevent striatal degeneration [37, 62]. A protocol we have employed (Table 8.1) was derived from the large experience of Morton with the glutaric aciduria of the Amish. Some have added insulin to the regimen and it is likely that we will as well. We use intravenous carnitine in a dose of 300 mg/kg. The initial dose for chronic oral carnitine administration approximates 100 mg/kg, and we adjust dosage dependent on intestinal tolerance and urinary carnitine ester excretion.

Implicit in programs of neonatal screening is the expectation that treatment will prevent encephalopathic neuronal damage. Experience to date suggests that this can be the case [10, 36, 37]. The occurrence of frontotemporal atrophy at birth implies restriction of any postnatal therapeutic effects, but there is even evidence that this too may improve [15]. In a recent report on 38 patients identified by neonatal screening and receiving intensive management, Hoffmann and colleagues [15] have written that encephalopathic crises were absent in 89 percent of these infants prospectively treated, while in a historical cohort 90 percent of patients developed encephalopathic crises [63]. Experience from the same group [64] indicates that some genotypes may lead to acute encephalopathy despite adherence to all of the current mainstays of treatment. An infant homozygous for E365K experienced such an episode and despite treatment was left with a dystonic, dyskinetic movement disorder, and characteristic striatal lesions on MRI [64].

A diet low in tryptophan and lysine will decrease in the excretion of glutaric acid in urine to one-third or more [1, 3, 20, 65] of the usual values, but clinical improvement resulting from diet alone has been little or none in patients who have had an encephalopathic crisis. Information from the international study [36] indicated clearly that in presymptomatic patients treatment is effective. The data indicated carnitine as effective in preventing secondary carnitine deficiency and in acting as a detoxifying agent. A lysine-restricted diet was found to be more effective than a protein-restricted diet, but it appears unlikely that a diet restricted in protein and supplements with a lysine tryptophan-free amino acid supplement would be any less effective, since the protein content of lysine in human food fed to infants does not change. An international working group recently published guidelines for the diagnosis and management of this disease [66]. The recommendation was for a diet restricted in lysine intake to the 'minimum requirements', which in our view are generous, plus supplementation with lysine-free, tryptophan-reduced amino acid mixtures and supplementation with carnitine.

Riboflavin, as the coenzyme of the dehydrogenase, has appeared logical, and 100–300 mg/day have been used [66–68], but also without clear evidence of therapeutic

Table 8.1 Management of acute imbalance in glutaric acidemia I

Time (hours)		mL/kg
0–1	Intravenous bolus 5% dextrose in Ringer lactate + 2 mEq/kg NaHCO ₃	20
1–24	Intravenous 12.5% dextrose 20 mEq/L KCl, 50 mEq/L NaHCO ₃ , 50 mEq/L NaCl; intravenous carnitine 300 mg/kg; insulin may be added in dosage of 0.05–0.1 µg/kg per hour monitoring blood glucose and electrolytes For vomiting, 0.15 mg/kg i.v. Zofran, may repeat in 4–8 hours. Alternatively, Kytril 10 µg/kg i.v. Calorimetry – provide CHO at least 1.5 × BMR	140

Patients have gastrostomy placed on diagnosis. Parents are instructed to stop protein and begin administration of calories and water on the way to the hospital.

effect. Riboflavin was pronounced ineffective by the international consortium [36, 66]. Low concentrations of GABA in the basal ganglia led to the use of the GABA analog 4-amino-3-(4-chlorophenyl)butyric acid (baclofen, Lioresal); results have usually not been impressive, but improvement was reported in two of three patients in a double-blind controlled study given 2 mg/kg per day [9]. Valproic acid has been recommended, but most feel this drug is contraindicated [3, 66]. Improvement has been reported clinically and in concentrations of GABA in the CSF following vigabatrine in doses of 35–50 mg/kg [69].

REFERENCES

- Goodman SI, Markey SP, Moe PG *et al.* Glutaric aciduria: a 'new' disorder of amino acid metabolism. *Biochem Med* 1975; **12**: 12.
- Iafolla AK, Kahler SG. Megalencephaly in the neonatal period as the initial manifestation of glutaric aciduria type I. *J Pediatr* 1989; **114**: 1004.
- Hoffmann GF, Trefz FK, Barth PG *et al.* Glutaryl-CoA dehydrogenase deficiency: a distinct encephalopathy. *Pediatrics* 1991; **88**: 1194.
- Hauser SE, Peters H. Glutaric aciduria type I: an underdiagnosed cause of encephalopathy and dystonia-dyskinesia syndrome in children. *J Pediatr Child Health* 1998; **34**: 302.
- Campistol J, Ribes A, Alvarez L *et al.* Glutaric aciduria type I: unusual biochemical presentation. *J Pediatr* 1992; **121**: 83.
- Nyhan WL, Zschocke J, Hoffmann G *et al.* Glutaryl-CoA dehydrogenase deficiency presenting as 3-hydroxyglutaric aciduria. *Mol Genet Metab* 1999; **66**: 199.
- Baric I, Wagner L, Feyh P *et al.* Sensitivity and specificity of free and total glutaric acid and 3-hydroxyglutaric acid measurements by stable-isotope dilution assays for the diagnosis for glutaric aciduria type I. *J Inherit Metab Dis* 1999; **22**: 867.
- Hoffman GF, Meier-Augenstein W, Nyhan WL. Physiology and pathophysiology of organic acids in cerebrospinal fluid. *J Inherit Metab Dis* 1993; **16**: 648.
- Smith WE, Millington DS, Koeber DD, Lesser PS. Glutaric acidemia, type I, missed by newborn screening in an infant with dystonia following promethazine administration. *Pediatrics* 2001; **107**: 1184.
- Soufi S, Rashed MS, Al Essa M *et al.* Glutaric acidemia type 1: first Saudi patient diagnosed by tandem mass spectrometry-based neonatal screening. *Ann Saudi Med* 1998; **18**: 160.
- Greenberg CR, Duncan AMV, Gregory CA *et al.* Assignment of human glutaryl-CoA dehydrogenase (GCDH) to the short arm of chromosome 19 (19p13.2) by in situ hybridization and somatic cell hybrid analysis. *Genomics* 1994; **21**: 289.
- Biery BJ, Stein DE, Morton DH, Goodman SI. Gene structure and mutations of glutaryl-coenzyme A dehydrogenase: impaired association of enzyme subunits due to an A421V substitution causes glutaric acidemia (type I) in the Amish. *Am J Hum Genet* 1996; **59**: 1006.
- Goodman SI, Stein DE, Schlesinger S *et al.* Glutaryl-CoA dehydrogenase mutations in glutaric acidemia (type 1): review and report of thirty novel mutations. *Hum Mutat* 1998; **12**: 141.
- Greenberg CR, Reimer D, Singal R *et al.* A G-to-T transversion at the +5 position of intron 1 in the glutaryl-CoA dehydrogenase gene is associated with the Island Lake variant of glutaric acidemia type 1. *Hum Mol Genet* 1995; **4**: 493.
- Hoffmann GF, Zschocke J. Glutaric aciduria type I: from clinical, biochemical and molecular diversity to successful therapy. *J Inherit Metab Dis* 1999; **22**: 381.
- Coates R, Rashed M, Rahbeeni Z *et al.* Glutaric aciduria type 1, first reported Saudi patient. *Ann Saudi Med* 1994; **14**: 316.
- Gregersen N, Brandt NJ, Christensen E *et al.* Glutaric aciduria: clinical and laboratory findings in two brothers. *J Pediatr* 1977; **90**: 740.
- Amir N, Elpeleg OBN, Shalev RS, Christensen E. Glutaric aciduria type I: enzymatic and neuroradiologic investigations of two kindreds. *J Pediatr* 1989; **90**: 983.
- Goodman SI, Norenberg M, Shikes RH *et al.* Glutaric aciduria: biochemical and morphologic considerations. *J Pediatr* 1977; **90**: 746.
- Floret D, Divry P, Dineon N, Monnet P. Acidurie glutarique: une nouvelle observation. *Arch Fr Pediatr* 1979; **36**: 462.
- Brandt NJ, Brandt S, Christensen E *et al.* Glutaric aciduria in progressive choreo-athetosis. *Clin Genet* 1978; **13**: 77.
- Kyllerman M, Steen G. Intermittently progressive dyskinetic syndrome in glutaric aciduria. *Neuropediatrics* 1977; **8**: 397.
- Dunger DB, Snodgrass GJAI. Glutaric aciduria type I presenting with hypoglycemia. *J Inherit Metab Dis* 1984; **7**: 122.
- Amir N, Elpeleg O, Shalev RS, Christensen E. Clinical heterogeneity and neuroradiologic features. *Neurology* 1987; **37**: 1654.
- Yager JY, McClarty BM, Seshia SS. CT-scan findings in an infant with glutaric aciduria type I. *Dev Med Child Neurol* 1988; **30**: 808.
- Wilson CJ, Collins JE, Leonard JV. Recurrent rhabdomyolysis in a child with glutaric aciduria type I. *J Inherit Metab Dis* 1999; **22**: 663.
- Hoffman GF, Trefz FK, Barth PG *et al.* Macrocephaly: an important indication for organic acid analysis. *J Inherit Metab Dis* 1991; **14**: 329.
- Brismar J, Ozand PT. CT and MRI of the brain in glutaric acidemia type I: a review of 59 published cases and a report of 5 new patients. *Am J Neuroradiol* 1995; **16**: 675.
- Leibel RL, Shih VE, Goodman SI *et al.* Glutaric acidemia: a metabolic disorder causing progressive choreoathetosis. *Neurology* 1980; **30**: 1163.
- Osaka H, Kimura S, Nezu A *et al.* Chronic subdural hematoma, as in initial manifestation of glutaric aciduria type-1. *Brain Dev* 1993; **15**: 125.
- Muntau AC, Röschinger W, Pfluger T *et al.* Subdurale Hygrome und Hämatome im Säuglingsalter als Initialmanifestation der glutarazidurie Typ I: Folgeschwere Fehldiagnose als Kindesmißhandlung. *Monatsschr Kinderh* 1997; **145**: 646.
- Drigo P, Burlina AB, Battistella PA. Subdural hematoma and glutaric aciduria type 1. *Brain Dev* 1993; **15**: 460.
- Woelfle J, Kreft B, Emons D, Haverkamp F. A diagnostic pitfall. *Pediatr Radiol* 1996; **26**: 779.

34. Chow CW, Haan EA, Goodman SI *et al.* Neuropathology in glutaric acidemia type I. *Acta Neuropathol* 1988; **76**: 590.
35. Soffer D, Amir N, Elpeleg ON *et al.* Striatal degeneration and spongy myelinopathy in glutaric acidemia. *J Neurol Sci* 1992; **107**: 199.
36. Kölker S, Garbade SF, Greenberg CR *et al.* Natural history, outcome, and treatment efficacy in children and adults with glutaryl-CoA dehydrogenase deficiency. *Pediatr Res* 2006; **59**: 840.
37. Strauss DJ, Shavelle RM, Anderson TW. Life expectancy of children with cerebral palsy. *Pediatr Neurol* 1998; **18**: 143.
38. Bjurgstad KB, Goodman SI, Freed CR. Age at symptom onset predicts severity of motor impairment and clinical outcome of glutaric acidemia type I. *J Pediatr* 2000; **137**: 681.
39. Goodman SI, Kohlhoff JG. Glutaric aciduria: inherited deficiency of glutaryl CoA dehydrogenase activity. *Biochem Med* 1975; **13**: 138.
40. Hyman DB, Tanaka K. Specific glutaryl-CoA dehydrogenating activity is deficient in cultured fibroblasts from glutaric aciduria patients. *J Clin Invest* 1984; **73**: 778.
41. Lenich AC, Goodman SI. The purification and characterization of glutaryl-coenzyme A dehydrogenase from porcine and human liver. *J Biol Chem* 1986; **261**: 4090.
42. Besrat A, Polan CE, Henderson LM. Mammalian metabolism of glutaric acid. *J Biol Chem* 1969; **244**: 1461.
43. Greenberg CR, Reimer D, Singal R. A G-to-T transversion at the +5 position of intron 1 in the glutaryl CoA dehydrogenase gene is associated with the Island Lake variant of glutaric acidemia type I. *Hum Mol Genet* 1995; **4**: 493.
44. Haworth JC, Booth FA, Coddle E *et al.* Phenotypic variability in glutaric aciduria type I: report of fourteen cases in five Canadian Indian kindreds. *J Pediatr* 1991; **118**: 52.
45. Busquets C, Merinero B, Christensen E *et al.* Glutaryl-CoA dehydrogenase deficiency in Spain: evidence of two groups of patients, genetically and biochemically distinct. *Pediatr Res* 2000; **48**: 315.
46. Zschocke J, Quak E, Guldberg P, Hoffmann GF. Mutation analysis in glutaric aciduria type I. *J Med Genet* 2000; **37**: 177.
47. Christensen E, Ribes A, Busquets C *et al.* Compound heterozygotes with R227P mutation on one allele in the glutaryl-CoA dehydrogenase gene is associated with no or very low glutarate excretion. *J Inherit Metab Dis* 1996; **48**: 95.
48. Anikster Y, Shaag A, Joseph A *et al.* Glutaric aciduria type I in the Arab and Jewish communities in Israel. *Am J Hum Genet* 1999; **59**: 1012.
49. Hellstrom B. Progressive dystonia and dyskinesia in childhood, a review of some recent advances. *Acta Paediatr Scand* 1982; **71**: 177.
50. Ribes A, Riudor E, Briones P *et al.* Significance of bound glutarate in the diagnosis of glutaric aciduria type I. *J Inherit Metab Dis* 1992; **15**: 367.
51. Wendel U, Bakkeren J, de Jong J, Bongaerts G. Glutaric aciduria mediated by gut bacteria. *J Inherit Metab Dis* 1995; **18**: 358.
52. Pitt J, Carpenter K, Wilcken B, Boneh A. 3-Hydroxyglutarate excretion is increased in ketotic patients: implications for glutaryl-CoA dehydrogenase deficiency testing. *J Inherit Metab Dis* 2002; **25**: 83.
53. Tortorelli S, Cuthbert CD, Tauscher A *et al.* The clinical significance of urine glutarylcarnitine for the biochemical diagnosis of glutaric acidemia type I. *Genet Med* 2003; May/June (Abstr. 45) 56.
54. Kyllerman M, Steen G. Glutaric aciduria. A 'common' metabolic disorder? *Arch Fr Pediatr* 1980; **37**: 279.
55. Lindner M, Kölker S, Schulze A *et al.* Neonatal screening for glutaryl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 2004; **27**: 851.
56. Morton DH, Bennett MJ, Seargeant LED *et al.* Glutaric aciduria type I: a common cause of episodic encephalopathy and spastic paralysis in the Amish of Lancaster County, Pennsylvania. *Am J Med Genet* 1991; **41**: 89.
57. Seargeant LED, Coddle E, Dialling LA *et al.* Carrier detection in glutaric aciduria type I using interleukin-2-independent cultured lymphocytes. *J Inherit Metab Dis* 1992; **15**: 733.
58. Goodman SI, Gallegos DA, Pullin CJ *et al.* Antenatal diagnosis of glutaric acidemia. *Am J Hum Genet* 1980; **32**: 695.
59. Stokke O, Goodman SI, Moe PG. Inhibition of brain glutamate decarboxylase by glutarate, glutaconate, and β -hydroxyglutarate: explanation of the symptoms in glutaric aciduria. *Clin Chim Acta* 1976; **66**: 411.
60. Ullrich K, Flott-Rahmel B, Schluff P *et al.* Glutaric aciduria type I: pathomechanisms of neurodegeneration. *J Inherit Metab Dis* 1999; **22**: 392.
61. de Mello CF, Kölker S, Ahlemeyer B *et al.* Intrastriatal administration of 3-hydroxyglutaric acid induces convulsions and striatal lesions in rats. *Brain Res* 2001; **1**: 70.
62. Hoffmann GF, Athanassopoulos S, Burlina AB *et al.* Clinical course, early diagnosis, treatment, and prevention of disease in glutaryl-CoA dehydrogenase deficiency. *Neuropediatrics* 1996; **27**: 115.
63. Kölker S, Garbade SF, Boy N *et al.* Decline of acute encephalopathic crises in children with glutaryl-CoA dehydrogenase deficiency identified by newborn screening in Germany. *Pediatr Res* 2007; **62**: 375.
64. Kölker S, Ramaekers VT, Zschocke J, Hoffmann GF. Acute encephalopathy despite early therapy in a patient with homozygosity for E365K in the glutaryl-coenzyme A dehydrogenase gene. *J Pediatr* 2001; **138**: 277.
65. Brandt NJ, Gregersen N, Christensen E *et al.* Treatment of glutaryl-CoA dehydrogenase deficiency (glutaric aciduria). *J Pediatr* 1979; **94**: 669.
66. Kölker S, Christensen E, Leonare JV *et al.* Guideline for the diagnosis and management of glutaryl-CoA dehydrogenase deficiency (glutaric aciduria type I). *J Inherit Metab Dis* 2007; **30**: 5.
67. Bennett MJ, Marlow N, Pollitt RJ, Wales JKH. Glutaric aciduria type I: biochemical investigations and postmortem findings. *Eur J Pediatr* 1986; **145**: 403.
68. Lipkin PH, Roe CR, Goodman SI, Batshaw ML. A case of glutaric acidemia type I: effect of riboflavin and carnitine. *J Pediatr* 1988; **112**: 62.
69. Francois B, Jaeken J, Gillia P. Vigabatrin in the treatment of glutaric aciduria type I. *J Inherit Metab Dis* 1990; **13**: 352.

3-Methylcrotonyl CoA carboxylase deficiency/ 3-methylcrotonyl glycinuria

Introduction	74	Treatment	77
Clinical abnormalities	75	References	77
Genetics and pathogenesis	76		

MAJOR PHENOTYPIC EXPRESSION

Reye-like episodes of ketoacidosis, hypoglycemia, hyperammonemia, and coma; seizures, failure to thrive, excretion of 3-methylcrotonylglycine and 3-hydroxyisovaleric acid; and deficiency of 3-methylcrotonyl CoA carboxylase. An increasing population of asymptomatic individuals, many of them adult women discovered because of elevated 3-hydroxyisovalerylcarnitine detected in the neonatal screening blood spots of their infants.

INTRODUCTION

3-Methylcrotonyl CoA carboxylase (EC 6.4.1.4) deficiency (Figure 9.1) is a rare disorder of leucine catabolism in which elevated quantities of 3-hydroxyisovaleric acid and 3-methylcrotonylglycine are found in the urine. The disorder is often referred to as isolated 3-methylcrotonyl CoA carboxylase deficiency, to distinguish it from

multiple carboxylase deficiency, as early reports and the majority of subsequent symptomatic patients with 3-methylcrotonylglycinuria had biotin-responsive multiple carboxylase deficiency as a consequence of deficiency of holocarboxylase synthetase (Chapter 4) or biotinidase (Chapter 5) [1, 2]. The disease was considered to be rare [3–11], until the development of programs of neonatal screening began turning up in so many patients that this

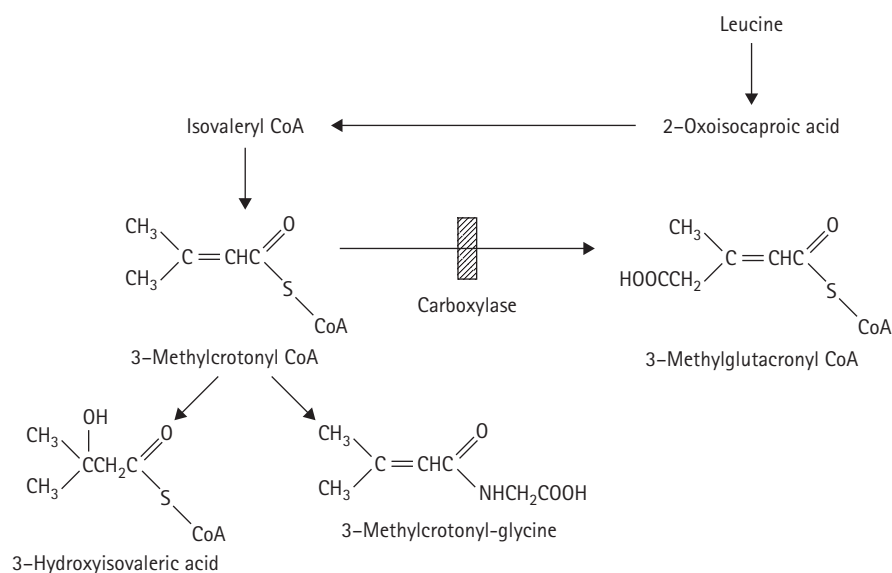


Figure 9.1 3-Methylcrotonyl CoA carboxylase, the site of the defect in 3-methylcrotonyl CoA carboxylase deficiency. The formation of the key metabolites results from hydration to 3-hydroxyisovaleric acid and conjugation with glycine.

disorder is being considered the most common of the organic acidemias [12]. In many instances of detection through newborn screening, it is the mother, not the newborn, who has 3-methylcrotonyl CoA carboxylase deficiency. The enzyme has two (MCC α and MCC β) subunits and the A and B genes have been cloned, and mutations in each have been defined [13–16].

CLINICAL ABNORMALITIES

The classic presentation has been relatively late in infancy, between one and three years of age, with an acute episode consistent with a diagnosis of Reye syndrome [11, 17]. The episode is classic for organic acidemia in that there is massive ketosis and systemic acidosis, leading to lethargy, coma, and even a fatal outcome [9]. Hypoglycemia may be prominent, symptomatic, and life-threatening [6, 11]. Death has also occurred from cerebral edema and cardiac arrest. There may be hyperammonemia and elevated levels of transaminases in blood. There may be microvesicular and macrovesicular deposition of fat in the liver [8, 9].

The onset of the initial episode may be with vomiting or convulsions. Between episodes, vomiting is uncommon and most patients appear completely well. Patients have noted subjectively that protein restriction led to general improvement, as well as a decrease in the number of exacerbations [3]. One patient had a neonatal onset of focal seizures and hypotonia, developed some developmental impairment and died in status epilepticus [18]. Hypotonia is commonly observed, and patients have been designated as having familial hypotonia and carnitine deficiency [17].

A number of patients have displayed quite a variety of clinical manifestations. One had chronic vomiting and failure to thrive. The onset of vomiting followed a graduation from human milk to conventional cow's milk-based formula at 3 weeks of life. In addition to vomiting, there was chronic diarrhea, numerous upper respiratory infections, a respiratory syncytial virus-induced bronchiolitis, and chronic mucocandidiasis. He had severe gastroesophageal reflux. Nevertheless, the existence of so many previously undiagnosed adults with the disease suggests that the general prognosis is good. In addition, most patients, once over the initial episode, have remained well and have been intellectually normal (Figure 9.2) [5, 19].

An increasing number of patients have been asymptomatic or very mildly symptomatic. Initially, these were patients diagnosed because they were siblings of patients and many never expressed symptoms of the disease [20, 21]. A sizeable number of recent patients has been detected through newborn screening. These include infants with 3-methylcrotonyl CoA carboxylase deficiency and adults discovered because their normal newly born infants failed the neonatal screening test for 3-hydroxyisovaleryl carnitine [12–14]. Some of these women have had myopathy or weakness, and carnitine



Figure 9.2 NB: A four-year-old girl with 3-methylcrotonyl CoA carboxylase deficiency. Her appearance and behavior have been quite normal for age. Her height was at the 25th percentile for age and the weight just below the 5th percentile. Muscle tone was reduced. She had always been a very fussy eater and ate very little. She has remained well and functions currently as an intelligent teenager. (Illustration was kindly provided by Dr Vivian Shin and the parents of the patient.)

deficiency, which could have been responsible for this symptomology [12]. Some also had elevated levels of uric acid and transaminases in the blood and histologic evidence of lipid deposits in the liver. These observations suggest that some of the nonspecific manifestations in earlier patients may have been unrelated to the underlying metabolic disorder. Nevertheless, the importance of the diagnosis is that any patient, regardless of even asymptomatic status, is at risk of the development, with the stress of infection, surgery, or a high protein load, of a typical Reye-like episode, which could be life-threatening. A patient who developed feeding difficulties and failure to gain weight at 11 weeks later developed seizures, spasticity, and fatal metabolic acidosis [22]. Another patient [23] had a metabolic stroke during an episode of hypoglycemia and metabolic imbalance coincident with a febrile illness. Following diagnosis and treatment, she was stable for five years of follow up, but hemiparesis and developmental delay remained. This adds to the list of metabolic diseases in which stroke-like episodes occur (Appendix). A patient detected by newborn screening whose parents were noncompliant with recommended management was well until 19 months, but then, following a respiratory infection, developed severe acidosis, hypoglycemia, and required intubation [24]. She was found to be carnitine deficient.

GENETICS AND PATHOGENESIS

The genetics of this disorder are autosomal recessive. Prenatal diagnosis should be possible by the assay of the enzyme in amniocytes or chorionic villus material [9, 25] or the direct gas chromatography-mass spectrometry (GCMS) determination of 3-hydroxyisovaleric acid in amniotic fluid [26]. Heterozygote detection may not be reliable, but values in fibroblasts, such as 21 and 42 percent of control activity, have been found in parents [9].

The molecular defect is in 3-methylcrotonyl CoA carboxylase (Figure 9.1). The diagnosis should be confirmed by the assay of the enzyme in leukocytes or cultured fibroblasts [3, 9, 24]. The other carboxylases for propionyl CoA and pyruvate should also be assayed, and so should biotinidase, because the distinction from multiple carboxylase deficiency is so important. A trial of biotin may be of interest, even though responsive patients with the isolated disease are rare (*vide infra*). The amounts of residual activity in fibroblasts may range from 0.05 to 3 percent in a single family [9], and up to as much as 12 percent [3]. Lymphocyte values may be much higher – approximately 46 percent of control in a patient in whom the mean fibroblast level was 10 percent [3]. Cultivation of cells in different levels of biotin does not usually affect activity. The enzyme has been purified from bovine kidney and rat liver and is an oligomer with two protein α and β subunits, like propionyl CoA carboxylase [27, 28]. Complementation studies [15] have shown clearly the presence of different A and B groups.

The genes for the α and β subunits have been cloned and sequenced independently by three different groups [13–15]. The A gene is located on chromosome 3q25–28 and has 19 exons. The B gene, on chromosome 5q12–13, has 17 exons. The genes encode proteins of 725 and 563 amino acids, respectively. A number of mutations has been defined, the majority of them missense.

Genotype–clinical phenotype correlations have been particularly elusive with this gene. Expression of missense mutations led to null or severely diminished MCC activity, while patients with these mutations varied from asymptomatic to acute neonatal presentations [29]. Some individuals with no detectable enzyme activity have been asymptomatic; yet some mutations are consistent with structural activity information on the enzyme. A missense mutation M325R led to absence of labeled biotin attached to the α subunit [14]. A missense mutation in the A gene and two in the B gene involved nonconservative substitutions of residues that are highly conserved in man, plants, and fungi. Construction of a null A gene in *Aspergillus* abolished the ability of this organism to grow on leucine as a sole carbon source [14].

An interesting molecular mechanism has led to dominant expression of a 3MCC gene mutation [30]. Two patients found to be heterozygous for R385S in the MCCA gene excreted elevated amounts of 3-hydroxyisovaleric acid and 3-methylcrotonylglycine. One had severe neurologic

sequelae of an episode of metabolic imbalance at three months; the other was found by newborn screening and was asymptomatic in infancy. In cotransfection experiments, insertion of the mild-type allele into a reference MCC defective cell line restored activity to 55 percent of control, while cotransfection of wild type with R385S restored activity only to 25 percent. This dominant negative effect is assumed to represent assembly of the varied protein into the normal multimeric enzyme.

The other interesting issue surrounding this mutation is that the patients have been responsive to biotin.

The accumulation of 3-methylcrotonylglycine behind the block in the carboxylase leads to the excretion of 3-hydroxyisovaleric acid and 3-methylcrotonylglycine (Table 9.1). The amounts are quite variable; usually but not always [3], the levels of the former are higher than the latter. An extreme example of this situation is two patients [31] with 3-methylcrotonyl CoA carboxylase deficiency in whom 3-methylcrotonylglyconuria was absent; both had 3-hydroxyisovaleric aciduria. Both had a mutation in the MCCB gene (V339M) and undetectable messenger from the other allele; 3-methylcrotonyl CoA is, after all, the immediate precursor behind the block. These experiences raise the possibility of a missed diagnosis if only GCMS organic acid analysis is used to make the diagnosis. A plasma acylcarnitine profile and/or enzyme assay should resolve this. Varying levels of the glycine conjugate, 3-methylcrotonylglycine, in different patients may reflect varying efficiency of glycine-N-acylase. Hydroxyisovalerylglycine has not been detected, presumably because the hydroxy acid is a poor substrate for glycine-N-acylase. Supplementation with glycine has been reported [23] not to increase the excretion of 3-methylcrotonylglycine. 3-Hydroxyisovalerylcarnitine has been identified in the urine [32] and identified as a product of leucine, and its occurrence in the blood has provided the basis for programs of neonatal screening. The identification of this carnitine ester provides evidence for the intramitochondrial origin of 3-hydroxyisovaleric acid via crotonase catalyzed conversion from 3-methylcrotonyl CoA and hydrolysis of the CoA ester. This contrasts with the microsomal origin of the compound in isovaleric acidemia in which 3-hydroxyisovalerylcarnitine is not found. It is important that 3-hydroxypropionic and methylcitric acids are not found in the urine. At the time of acute ketotic illness, 3-hydroxybutyric acid, acetoacetic acid, and dicarboxylic acids are found on organic acid

Table 9.1 Urinary excretion of the key metabolites

Metabolite	Range of excretion (mmol/mol creatinine)
3-Hydroxyisovaleric acid	100–60,000
3-Methylcrotonylglycine	70–5200

analysis. 2-Oxoglutaric acid excretion may be elevated and 3-methylcrotonylglutamic acid has been found [10].

Concentrations of free-carnitine in the blood may be very low and the excretion of carnitine esters is high.

The development of tandem mass spectrometry and assay of carnitine esters of CoA containing organic acids has led to highly effective programs of expanded neonatal screening. These programs have given, for the first time, reliable data on the prevalence of 3-methylcrotonylCoA carboxylase deficiency. The incidence in the population of North Carolina was reported as one in 52,000 [33]. In Australia, incidences of one in 27,000 [34] and one in 110,000 [35] have been reported. The incidence in Bavaria was one in 30,000 [36]. This is another metabolic disease that appears to be common in the Amish-Mennonite populations of the United States [12]. In data from Bavaria, less than 10 percent of patients detected by screening and found to have mutations were found to develop symptoms [37]. In addition, it was concluded that none of the symptoms reported could clearly be attributed to deficiency in this enzyme. This appears to be incompatible with the fact that patients with this disease have been observed who have had clearcut organic acidemia presentations with ketoacidosis [24]. It is increasingly clear that this number is small. Nevertheless, on the basis of the Bavarian data, infants in Germany are no longer screened for this disease.

Among infants found to have elevated C50Hcarnitine via newborn screening, a certain number of infants turn out to be normal products of an asymptomatic mother with MCC deficiency. In general, the levels of C50Hcarnitine tend to be higher in this situation than when they are provided by an affected infant. Elevated C50Hcarnitine is also found in holocarboxylase synthetase (Chapter 5) and in 3-hydroxy 3-methylglutarylCoA lyase deficiency (Chapter 45), as well as in 2-oxothiolase deficiency (Chapter 13) where the elevated 2-methyl-2-hydroxybutyrylcarnitine shares the same mass as 3-hydroxyisovaleryl carnitine.

TREATMENT

Modest restriction of the intake of protein and a modest supplement of carnitine (100 mg/kg) are adequate to prevent most further evidence of disease, once the diagnosis is made, assuming compliance [24]. Generally, the protein intake prescribed has been from 1.5 to 2.0 g/kg per day [5–8, 23]. A protein-free source of calories, vitamins, and minerals, such as Profree (Ross), may be useful. Alternatively, low protein intake may be supplemented by a leucine-free medical food (Analog, Maxamaid Xleu, Ross) [37–39]. We have not generally employed these in this disease. Computer programs are available [37] to aid in the preparation of diets. Recommended intake of leucine has ranged from 60 to 100 mg/kg in infants under six months, and 30–60 mg/kg in children over seven years. Carnitine therapy should be designed to restore plasma

concentrations of free-carnitine and to achieve maximum excretion of carnitine esters, within the range of intestinal intolerance.

The acute ketoacidotic episode is treated as in classical organic acidemia with large amounts of water and electrolyte containing bicarbonate (Chapter 1) and intravenous carnitine (300 mg/kg). If prolonged parenteral nutrition is required, formulations have been designed that exclude leucine [40]. These can be supplemented with standard parenteral solutions of amino acids, so that total restriction of any individual amino acid is not pursued for more than a few days.

REFERENCES

1. Sweetman L, Nyhan WL. Inheritable biotin-treatable disorders and associated phenomena. *Annu Rev Nutr* 1986; **6**: 317.
2. Leonard JV, Seakins JW, Bartlett K *et al*. Inherited disorders of 3-methylcrotonyl CoA carboxylation. *Arch Dis Child* 1981; **56**: 53.
3. Tuchman M, Berry SA, Thuy LP, Nyhan WL. Partial methylcrotonyl-coenzyme A carboxylase deficiency in an infant with failure to thrive, gastrointestinal dysfunction, and hypertonia. *Pediatrics* 1993; **91**: 664.
4. Finnie MDA, Cottrel K, Seakins JWT *et al*. Massive excretion of 2-oxoglutaric acid and 3-hydroxyisovaleric acid in a patient with deficiency of 3-methylcrotonyl-CoA carboxylase. *Clin Chim Acta* 1976; **95**: 513.
5. Beemer FA, Bartlett K, Duran M *et al*. Isolated biotin-resistant 3-methylcrotonyl-CoA carboxylase deficiency in two sibs. *Eur J Pediatr* 1982; **138**: 351.
6. Bartlett K, Bennett MJ, Hill RP *et al*. Isolated biotin-resistant-3-methylcrotonyl CoA carboxylase deficiency presenting with life threatening hypoglycemia. *J Inherit Metab Dis* 1984; **7**: 182.
7. Tsai MY, Johnson DD, Sweetman L *et al*. Two siblings with biotin-resistant 3-methylcrotonyl-coenzyme A carboxylase deficiency. *J Pediatr* 1989; **115**: 110.
8. Layward EM, Tanner MS, Politt RJ *et al*. Isolated biotin-resistant 3-methylcrotonyl-CoA carboxylase deficiency presenting as a Reye syndrome-like illness. *J Inherit Metab Dis* 1989; **12**: 339.
9. Kobori JA, Johnston K, Sweetman L. Isolated 3-methylcrotonyl CoA carboxylase deficiency presenting as a Reye-like syndrome. *Pediatr Res* 1989; **25**: 142A.
10. Rolland MO, Divry P, Zabot MT *et al*. Isolated 3-methylcrotonyl-CoA carboxylase deficiency in a 16-month-old child. *J Inherit Metab Dis* 1991; **14**: 838.
11. Gitzelmann R. Isolated (biotin-resistant) 3-methylcrotonyl-CoA carboxylase deficiency presenting with life-threatening hypoglycemia. *J Inherit Metab Dis* 1987; **10**: 290.
12. Gibson KM, Bennett MJ, Naylor EW, Morton DH. 3-Methylcrotonyl-coenzyme A carboxylase deficiency in Amish/Mennonite adults identified by detection of increased acylcarnitines in blood spots of their children. *J Pediatr* 1998; **132**: 519.

13. Holzinger A, Roschinger W, Lagler F *et al.* Cloning of the human MCCA and MCCB genes and mutations therein reveal the molecular cause of 3-methylcrotonyl-CoA: carboxylase deficiency. *Hum Molec Genet* 2001; **10**: 1299.
14. Gallardo ME, Resviate LR, Rodriguez JM *et al.* The molecular basis of 3-methylcrotonylglycinuria, a disorder of leucine catabolism. *Am J Hum Genet* 2001; **68**: 334.
15. Baumgartner MR, Almashanu S, Sourmala T *et al.* The molecular basis of human 3-methylcrotonyl-CoA carboxylase deficiency. *J Clin Invest* 2001; **107**: 495.
16. Dantas FM, Suormala T, Randolph A *et al.* 3-Methylcrotonyl-CoA carboxylase deficiency: mutation analysis in 28 probands, 9 symptomatic and 19 detected by newborn screening. *Hum Mutat* 2005; **26**: 164.
17. Elpeleg ON, Hawkin S, Barash V *et al.* Isolated biotin-resistant 3-methylcrotonyl-CoA carboxylase presenting as a clinically severe form in a newborn with fatal outcome. *J Inherit Metab Dis* 1992; **15**: 863.
18. Bannwart C, Wermuth B, Baumgartner R *et al.* Isolated biotin-resistant 3-methylcrotonyl-CoA carboxylase presenting as a clinically severe form in a newborn with fatal outcome. *J Inherit Metab Dis* 1992; **15**: 863.
19. Leonard JV, Daish P, Naughten ER, Bartlett K. The management and long term outcome of organic acidemias. *J Inherit Metab Dis* 1984; **7**: 13.
20. Mourmans J, Bakkersen J, de Jong J *et al.* Isolated (biotin-resistant) 3-methylcrotonyl-CoA carboxylase deficiency: four sibs devoid of pathology. *J Inherit Metab Dis* 1995; **18**: 643.
21. Pearson MA, Aleck KA, Heidenreich RA. Benign clinical presentation of 3-methylcrotonylglycinuria. *J Inherit Metab Dis* 1995; **18**: 640.
22. Finnie MD, Cottrill K, Seakins JW, Snedden W. Massive excretion of 2-oxoglutaric acid and 3-hydroxyisovaleric acid in a patient with a deficiency of 3-methylcrotonyl-CoA carboxylase. *Clin Chim Acta* 1976; **73**: 513.
23. Steen C, Baumgartner ER, Duran M *et al.* Metabolic stroke in isolated 3-methylcrotonyl-CoA carboxylase deficiency. *Eur J Pediatr* 1996; **158**: 730.
24. Ficicioglu C, Payan I. 3-Methylcrotonyl-CoA carboxylase deficiency: metabolic decompensation in a noncompliant child detected through newborn screening. *Pediatrics* 2006; **118**: 2555.
25. Weyler W, Sweetman L, Maggio DC, Nyhan WL. Deficiency of propionyl-CoA carboxylase in a patient with methylcrotonylglycinuria. *Clin Chim Acta* 1977; **76**: 321.
26. Jakobs C, Sweetman L, Nyhan WL, Packman S. Stable isotope dilution analysis of 3-hydroxyisovaleric acid in amniotic fluid: contribution to the prenatal diagnosis of inherited disorders of leucine catabolism. *J Inherit Metab Dis* 1984; **7**: 15.
27. Lau EP, Cochran BC, Munson L, Fall RR. Bovine kidney-3-methylcrotonyl-CoA and propionyl-CoA-carboxylases: each enzyme contains non-identical subunits. *Proc Natl Acad Sci USA* 1979; **76**: 214.
28. Oei J, Robinson BH. Simultaneous preparation of the three biotin-containing mitochondrial carboxylases from rat liver. *Biochim Biophys Acta* 1985; **840**: 1.
29. Desviat LR, Perez-Cerda C, Perez B *et al.* Functional analysis of MCCA and MCCB mutations causing methylcrotonylglycinuria. *Mol Gen Metab* 2003; **80**: 315.
30. Baumgartner M, Dantas MF, Suormala T *et al.* Isolated 3-methylcrotonyl-CoA carboxylase deficiency: evidence for an allele-specific dominant negative effect and responsiveness to biotin therapy. *Am J Hum Genet* 2004; **75**: 790.
31. Wolfe LA, Finegold DN, Vockley J *et al.* Potential misdiagnosis of 3-methylcrotonyl-coenzyme A carboxylase deficiency associated with absent or trace urinary 3-methylcrotonylglycine. *Pediatrics* 2007; **120**: e1335.
32. van Hove JLK, Rutledge SL, Nasa MA *et al.* 3-Hydroxyisovalerylcarnitine in 3-methylcrotonyl-CoA carboxylase deficiency. *J Inherit Metab Dis* 1995; **18**: 592.
33. Smith WE, Muenzer J, Frazier D *et al.* Elevation of elevated hydroxyisovalerylcarnitine in the newborn screen by tandem mass spectrometry. *Am J Hum Genet* 2000; **67**(Suppl. 2): 292.
34. Ranieri E, Gerace R, Barlett B *et al.* The introduction of tandem mass spectrometry in to the South Australian neonatal screening program: benefits and costs. *J Inherit Metab Dis* 2000a; **23**(Suppl. 1): 006 (poster).
35. Wilcken B, Wiley V, Carpenter K. Two years of routine newborn screening by tandem mass spectrometry (MSMS) in New South Wales, Australia. *J Inherit Metab Dis* 2000; **23**(Suppl. 1): 007 (poster).
36. Roscher A, Liebl B, Fingerhut R, Olgemoller B. Prospective study of MS-MS newborn screening in Bavaria, Germany: interim results. *J Inherit Metab Dis* 2000; **23**(Suppl. 1): 008 (poster).
37. Stadler SC, Polanetz R, Maier M *et al.* Newborn screening for 3-methylcrotonyl-CoA carboxylase deficiency: population heterogeneity of MCCA and MNCCB mutations and impact on risk assessment. *Hum Mutat* 2006; **27**: 748.
38. Acosta PB. *The Ross Metabolic Formula System Nutrition Support Protocols*. Columbus, OH: Ross Laboratories, Appendices A, G, and I, 1989.
39. Elsas LJ II, Acosta PB. Nutrition support of inherited metabolic diseases. In: Shils ME, Young VR (eds). *Modern Nutrition in Health and Disease*, 7th edn. Philadelphia, PA: Lea & Febiger, 1988: 1337.
40. Nyhan WL, Rice-Asaro M, Acosta P. Advances in the treatment of amino acid and organic acid disorders. In: Desnick RJ (ed.). *Treatment of Genetic Diseases*. New York: Churchill Livingstone, 1991: 45.

D-2-hydroxyglutaric aciduria

Introduction	79	Treatment	83
Clinical abnormalities	79	References	83
Genetics and pathogenesis	81		

MAJOR PHENOTYPIC EXPRESSION

Developmental delay, macrocephaly, seizures, vomiting, cerebral atrophy, D-2-hydroxyglutaric aciduria, and defective activity of D-2-hydroxyglutarate dehydrogenase (type 1) or gain of function mutations in IDH₂ the gene for isocitrate dehydrogenase (type 2).

INTRODUCTION

D-2-hydroxyglutaric aciduria is an organic aciduria in which the clinical phenotypic spectrum is quite broad [1–7]. It has ranged from asymptomatic to severely affected. 2-Hydroxyglutaric aciduria is identifiable by systems of gas chromatography-mass spectrometry (GCMS) organic acid analysis of the urine, but it is critical that the optical isomeric form be determined, because D-2-hydroxyglutaric aciduria and L-2-hydroxyglutaric aciduria ([Chapter 11](#)) are quite distinct diseases.

In 2004, Achouri and colleagues [8] identified a D-2-hydroxyglutarate dehydrogenase ([Figure 10.1](#)) in rat liver. This mitochondrial enzyme catalyzes the conversion of D-2-hydroxyglutaric acid to 2-ketoglutaric acid ([Figure 10.1](#)). Mutation analyses by Struys and colleagues [9] led to the recognition that mutations in the dehydrogenase gene are found in patients with both mild and severe clinical disease. Kranendijk *et al.* [10] have now shown clearly that there are two populations of patients with D-2-hydroxyglutaric aciduria. They referred to as type I those patients who had defective activity of the dehydrogenase enzyme and mutations in the gene. Oddly, the other group, type 2 which had neither mutations nor defective activity of the enzyme had higher levels of D-2-hydroxyglutaric acid in body fluids. These patients have now been shown to have gain of function mutations in the gene IDH₂ for isocitrate dehydrogenase.

CLINICAL ABNORMALITIES

Developmental impairment is a common feature of the disease. In 17 patients with the severe phenotype, manifestations were early onset: at seven months, one [1] could not sit or roll and did not fix or follow; another [3] was cortically blind. Most of the patients classified as severe [4, 5] had little evidence of mental development. Among the patients with milder presentations, mental impairment and hypotonia were the rule, although the younger sister of one patient appeared by three years to have only speech delay, and both sisters were dysmorphic, suggesting the possibility of another etiology for the mental impairment [1].

The clinical phenotype was set out by van der Knaap and associates [4] in an international survey of 25 patients with documented D-2-hydroxyglutaric aciduria. The first symptom may be vomiting. In three patients [1, 5], it was sufficiently severe that a diagnosis of pyloric stenosis was made and a pyloromyotomy performed. A number of metabolic diseases, particularly organic acidemias that present in the neonatal period, may be diagnosed as pyloric stenosis or similar surgical disease ([Appendix](#)).

Macrocephaly may be another early symptom ([Figure 10.2](#)) [1]. At seven months, the head circumference in one patient at 47 cm was in the 50th percentile for 19 months. This patient also had chronic subdural collections of fluid. Macrocephaly was also present in three of the patients

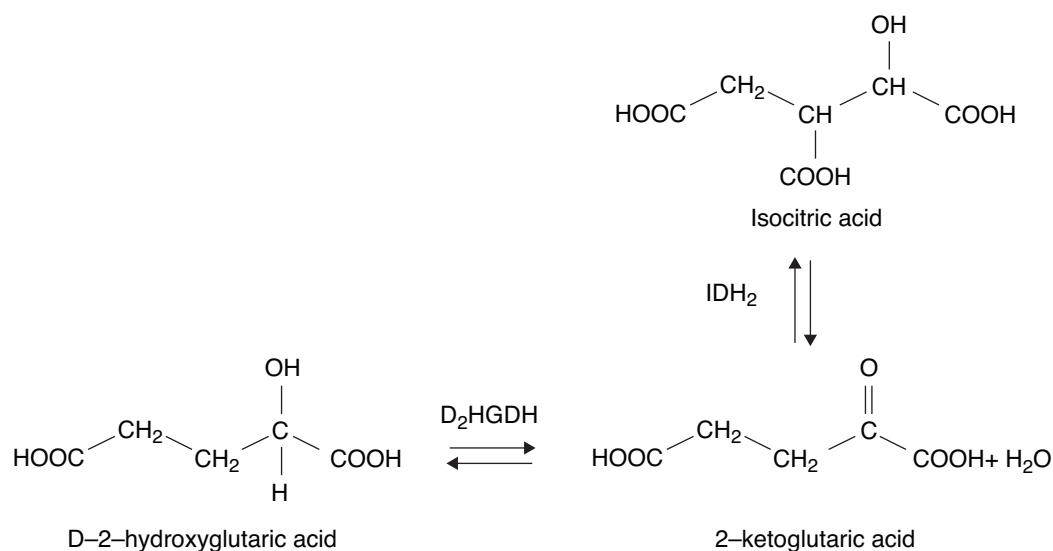


Figure 10.1 D-2-hydroxyglutarate dehydrogenase (D-2-HGH), the molecular defect in D-2-hydroxyglutaric aciduria and isocitrate dehydrogenase (IDH₂) the newly described defect.

classified as mild [5]; and four of the severe patients developed microcephaly. Macrocephaly and subdural collections of fluid are also characteristic of glutaric aciduria resulting from glutarylCoA dehydrogenase deficiency (Chapter 8), and these diseases should be considered (Appendix) before a diagnosis of nonaccidental trauma is made.

Seizures may be grand mal or myoclonic; some were of neonatal onset and abnormalities [1, 3] of the electroencephalogram (EEG) included hypsarrhythmia [1]. The concentration of protein in the cerebrospinal fluid may be increased [1]. Cerebral blindness or delayed cerebral visual development has been observed [3–5].

Involuntary movements described have included chorea, dystonic posturing, and episodic opisthotonic arching and extensor posturing [1, 3, 5]. Hypotonia has been observed in a number of patients [1, 3, 5], but there may also be hypertonia. Irritability and lethargy have been observed. Spasticity, increased deep tendon reflexes, and positive Babinski responses have been present [1, 5].

Cardiomyopathy was found in a number of the severely affected patients, in some of whom it was clinically symptomatic. It has been dilated as well as hypertrophic. In some patients, cardiomegaly was evident only by ultrasound. One patient had a ventricular septal defect and one a mild coarctation of the aorta and hypertrophy of the left ventricle. A patient with severe disease displayed respiratory distress and died at ten months of cardiogenic shock as a result of cardiomyopathy [11]. Other patients have had stridor or apnea and one required tracheostomy [4].

A variety of dysmorphic features have been noted in patients with this disease, including plagiocephaly, asymmetric ears, transverse palmar creases, epicanthal folds, a frontal upsweep of the hair and coarse features

[1]. Facial dysmorphism has been particularly common, including a flat face, a broad nasal bridge, and abnormalities of the external ears [12]. Spondyloenchondromatosis has been reported in three patients, all products of consanguineous matings [13–15]. A pair of monozygotic twins had malar flattening and a broad nasal root [16]. One had severe developmental delay, which worsened after an infantile rotovirus infection, and she was born with esophageal atresia and a tracheoesophageal fistula. Her twin had macrocephaly, but had normal development. A girl with D-2-hydroxyglutaric aciduria had turricephaly, brachycephaly, a broad flat face with coarse features and a prominent jaw [17]. Two patients with massive elevations of D-2-hydroxyglutaric acid were asymptomatic [18].

Imaging of the central nervous system with magnetic resonance (MR) or computed tomography (CT) has regularly revealed cerebral atrophy with consistent enlargement of the lateral ventricles [1, 3–5, 16, 19]. White matter atrophy was progressive. Disease duration was significantly longer in patients with white matter atrophy than those without in a survey of 56 patients [19]. White matter abnormalities affected primarily the frontal and subcortical areas. Early changes were often those of a mildly swollen appearance, at least partially multifocal. The periventricular rim was relatively spared. Bilateral signal intensity abnormalities in the basal ganglia became more diffuse as cerebral white matter atrophy progressed.

Subdural effusions have been found in four patients [5]. One 14-month-old developed acute left-sided hemiparesis and a right middle cerebral artery infarction, and later a left striatal infarction, and finally infarction of the left anterior, middle, and posterior cerebral arteries followed by disappearance of the left hemisphere [4]. Another patient had multiple aneurisms of the middle



Figure 10.2 AF: A 19-month-old patient with D-2-hydroxyglutaric aciduria. (A) She had macrocephaly, deeply recessed orbits, epicanthal folds, a wide nasal bridge, and an upturned nose. There were micrognathia and a carp-shaped mouth. (B) She could not sit without support. There was frontal and occipital bossing. (C) The curvature of the spine is an index of the marked hypotonia even when supported while sitting.

cerebral arteries bilaterally. A patient with severe disease who died at ten months had on magnetic resonance imaging (MRI) increased T_2 signal in the substantia nigra, caudate, and thalamus, lesions similar to those of patients with mitochondrial disease [11]. The infant with tracheoesophageal fistula [16] had early loss of cerebral volume, and hyperintense signal in the basal ganglia followed by dense calcification. Another infant had absence of the corpus callosum [20]. This patient also had multiple intracranial hemorrhages. Absence of the corpus callosum was also reported in two other patients with D-2-hydroxyglutaric aciduria [12, 21].

GENETICS AND PATHOGENESIS

The genetic transmission of the disorder is autosomal recessive. Affected offspring of normal parents have been observed [1, 3, 22], as has consanguinity. Prenatal diagnosis

of an affected fetus has been made by the analysis of D-2-hydroxyglutaric acid in amniotic fluid by selective ion-monitoring GCMS with stable isotope dilution internal standard [22]. In the amniotic fluid of affected fetuses, the compound was elevated ten-fold [3, 17]. In a family in which the mutation is known, analysis of the DNA may be employed in prenatal diagnosis. Genetic counseling is complicated by the occurrence of patients with normal development [16, 22].

The biochemical hallmark of the disease is the accumulation of D-2-hydroxyglutaric acid in body fluids. The compound is readily detected and quantified by organic acid analysis of the urine. GCMS does not distinguish the enantiomers, D and L-2-hydroxyglutaric acids. The chiral center at the asymmetric second carbon results in differential rotation of polarized light shone on the two compounds. Light is rotated to the right by the D (Latin, *dexter*) form and to the left by the L (Latin, *laevus*) compound. The terms R and S are sometimes used for

chirality of D and L, respectively. The identification as the D-form is accomplished by chemical ionization GCMS of the O-acetyl-di-2-butyl ester [22]. A more recently reported method [23] utilized R, R-diacetyltartaric anhydride as the agent, separated the derivatives by liquid chromatography and quantified by tandem mass spectrometry (MS/MS) [23, 24]. Urinary excretion of D-2-hydroxyglutaric acid ranged from 18 (this patient also recorded a level of 1072) to 7076 mmol/mol creatinine [1, 2, 5]. Control individuals excreted 3–17 mmol/mol creatinine. In our patient, the concentration in the cerebrospinal fluid (CSF) of 313 $\mu\text{mol/L}$ was slightly higher than that of the plasma (283 $\mu\text{mol/L}$) [1], while in another patient [2, 3] the plasma concentration of 62 $\mu\text{mol/L}$ was slightly greater than that of the CSF (25 $\mu\text{mol/L}$). Overall, the CSF level was higher than that of the plasma in all but one patient [5].

Excretion of L-2-hydroxyglutaric acid was normal in all but one patient [5]. There was no relationship between the level of D-2-hydroxyglutaric acid in urine, plasma, or CSF and the severity of disease. One patient with severe disease had intermittently normal and high levels of excretion [11].

Excretion of 2-oxoglutaric acid ranged from 404 to 862 mmol/mol creatinine [1], amounts similar to those reported in 2-oxoglutaric aciduria [25]. This was found in other patients [5], and other citric acid cycle compounds were up in some, usually to a lesser level [5]. The excretion of 2-oxoglutaric acid normally decreases with age, and decrease toward normal has been observed in two patients [2], while their excretion of D-2-hydroxyglutaric acid remained high. Elevated concentrations of 4-aminobutyric acid (GABA) were found in the CSF in almost all of the patients studied [5]. Levels of 20 and 28 $\mu\text{mol/L}$ have been reported [1, 3]. One patient had an increased amount of glycine in the urine. Decreased levels of carnitine have been found in many patients, but many have been receiving valproate [5]. Acylcarnitine profiles have been normal, but one had multiple elevations, as seen in multiple acylCoA dehydrogenase deficiency, but without excretion of glutaric and ethylmalonic acids. Increased levels of lactic acid in the urine were found in a few patients [5, 11].

In studies of cultured fibroblasts [26], the media in which cells derived from patients with D-2-hydroxyglutaric aciduria grew contained 5–30 times the control concentration of D-2-hydroxyglutaric acid. Studies of cultured human lymphoblasts incubated with ^{13}C -labeled glucose or ^3H -labeled glutamate indicated that D-2-hydroxyglutaric acid is rapidly converted to 2-oxoglutaric acid [27]. D-2-hydroxyglutaric acid is a metabolic intermediate in a variety of pathways. The simplest conversion from 2-oxoglutarate is catalyzed by D-2-hydroxyglutaric acid dehydrogenase (EC 1.1.99.6). This is the site of one defect [28]. Mean activities in control fibroblast and lymphoblast homogenates were 208 ± 207 and 1670 ± 940 pmol/hour/mg protein. Cells derived from patients were less than 41 pmol/hour/mg protein.

The reaction is also catalyzed by a transhydrogenase and in the exchange 4-hydroxybutyric acid is converted

to succinic semialdehyde [29]. Succinic semialdehyde is the immediate catabolic product of 4-aminobutyric (GABA), and thus interference with this pathway and accumulation of GABA would be expected to have neurologic consequences, as in the case of GABA transaminase deficiency [30].

The molecular defect in the activity of D-2-hydroxyglutarate dehydrogenase (Figure 10.1) interferes with the conversion of hydroxyglutarate to 2-oxoglutarate. The structure of the enzyme is homologous to that of D-lactate dehydrogenase [8] and by analogy it is thought to transfer its electron to electron transfer flavoprotein (ETF). The enzyme is highly active in liver and kidney, but it is also active in brain and heart.

Two patients with severe disease were found to have mutations in the dehydrogenase gene [7]. One was heterozygous for two mutations in intron 1 (IVS1-23-A \rightarrow G) and a missense mutation in exon 2 (c.440T \rightarrow G) which resulted in a substitution of serine for isoleucine (Ile 147 Ser). The intronic mutation created an alternative splice-acceptor site leading to a frame shift and premature stop. Mutations were also found in two unrelated consanguineous Palestinian families; in one of which two affected siblings were asymptomatic; in the other, the patient had mild disease consisting of absence seizures readily controlled, difficulty reading, hyperactivity, and behavior problems [9]. In one family, the affected sibs were homozygous for an A to G transition in intron 4 (IVS4-2A \rightarrow G). In the other family, the patient was homozygous for an A to G transition at nucleotide 1315 which changed asparagine 439 to aspartic acid. Expression of this mutation led to an enzyme with 13 percent of control activity. The mutation in the first family led to an unstable mRNA. In both families, each parent was heterozygous.

The twins with the very different phenotypes were compound heterozygotes for c.326-327dupTC in exon 2 and c1123G \rightarrow T in exon 7 [16]. The duplication resulted in a frame shift which substituted arginine by glutamic acid at 110 and termination of the 19th codon. The missense mutation substituted tyrosine for aspartic acid 375. Each parent was heterozygous for one mutation. The unusual differences in the phenotype make genotype/phenotype correlations problematic. Twinning may have had something to do with it.

In the most recent assessment [10], 29 mutations were found, 21 novel, each of which predicted a truncated protein. Molecular genetic abnormalities were found in all patients in whom deficiency of enzyme activity was documented. However, neither was found in over 50 percent of patients.

The key to the molecular defect in type 2 patients came from observations in cancer cells in which D-2-hydroxyglutarate accumulated in cells with superactivity of isocitrate dehydrogenase. Two genes IDH_1 and IDH_2 code for isocitrate dehydrogenase (Figure 10.1). Fifteen patients were found to have gain of function mutations in IDH_2 [31]. It is recommended in the work up of patients in

whom 2-hydroxyglutarate is found on organic acid analysis that first the optical rotation D or L is determined. In those with the D form, mutational analysis is carried out on D-2-HGH and IDH₂.

The product 4-hydroxybutyric acid is also neuropharmacologically active, as illustrated by patients with 4-hydroxybutyric aciduria which is due to succinic semialdehyde dehydrogenase deficiency (Chapter 12). The elevated levels of GABA in the CSF in patients with D-2-hydroxyglutaric aciduria would be consistent with abnormalities in this pathway. Fibroblasts derived from patients with D-2-hydroxyglutaric aciduria have been found to have normal transhydrogenase activity; on the other hand, it is likely that this enzyme is responsible for the occurrence of D-2-hydroxyglutaric aciduria in patients with 4-hydroxybutyric aciduria (Chapter 12). In patients with multiple acylCoA dehydrogenase deficiency (glutaric aciduria type II) (Chapter 44) [32], 2-hydroxyglutaric acid excretion is elevated and it is the D-isomer that is predominant. Of course, in glutaric aciduria type II, any hydroxyl acid accumulated might lead to the formation of D-2-hydroxyglutaric aciduria in the presence of 2-oxoglutarate in a transhydrogenase reaction [33].

The pathophysiology of the disease is thought to represent a developmental neurotoxicity of D-2-hydroxyglutaric acid. Incubation of pathologic concentrations of the compound with primary neuronal cultures from chickens and rats led to excitotoxic cell damage via activation of the N-methyl-D-aspartic acid receptor [34]. D-2-hydroxyglutaric acid inhibited creatine kinase [34] in brain and in skeletal and cardiac muscle, and cytochrome-c oxidase activity in fibroblasts *in vitro*, but electron transport chain activity in the fibroblasts of patients was normal. D-2-hydroxyglutaric acid was also found to inhibit *in vitro* the activity of cytochrome oxidase in rat brain fractions [11].

TREATMENT

Approaches to treatment have not been developed. The recently documented heterogeneity adds complexity. On the other hand, establishment of the molecular nature of the gene and enzyme represent major additions to the understanding of this disease.

REFERENCES

1. Nyhan WL, Shelton GD, Jakobs C *et al.* D-2-hydroxyglutaric aciduria. *J Child Neurol* 1995; **10**: 132.
2. Gibson KM, Craigen W, Herman GE, Jakobs C. D-2-hydroxyglutaric aciduria in a newborn with neurologic abnormalities: a new metabolic disorder? *J Inherit Metab Dis* 1993; **16**: 490.
3. Craigen WJ, Sekul EA, Levy MI *et al.* D-2-hydroxyglutaric aciduria in a neonate with seizures and central nervous system dysfunction. *Pediatr Neurol* 1994; **10**: 49.
4. van der Knaap MS, Jakobs C, Hoffmann GF *et al.* D-2-hydroxyglutaric aciduria: Further clinical delineation. *J Inherit Metab Dis* 1999; **22**: 404.
5. van der Knaap MS, Jakobs C, Hoffmann GF *et al.* D-2-hydroxyglutaric aciduria: Biochemical marker or clinical disease entity? *Ann Neurol* 1999; **45**: 111.
6. Korman SH, Salomons GS, Gutman A *et al.* D-2-hydroxyglutaric aciduria and glutaric aciduria type 1 in siblings: coincidence, or linked disorders? *Neuropediatrics* 2004; **35**: 151.
7. Struys EA, Korman SH, Salomons GS *et al.* Mutations in phenotypically mild D-2-hydroxyglutaric aciduria. *Ann Neurol* 2005; **58**: 626.
8. Achouri Y, Noel G, Vertommen D *et al.* Identification of a dehydrogenase acting on D-2-hydroxyglutarate. *Biochem J* 2004; **381**: 35.
9. Struys EA, Salomons GS, Achouri Y *et al.* Mutations in the D-2-hydroxyglutarate dehydrogenase gene cause D-2-hydroxyglutaric aciduria. *Am J Hum Genet* 2005; **76**: 358.
10. Kranendijk M, Struys EA, Gibson KM *et al.* Evidence for genetic heterogeneity in D-2-hydroxyglutaric aciduria. *Hum Mutat* 2010; **31**: 279.
11. Wajner M, Vargas CR, Funayama C *et al.* D-2-hydroxyglutaric aciduria in a patient with a severe clinical phenotype and unusual MRI findings. *J Inherit Metab Dis* 2002; **25**: 28.
12. Amiel J, DeLonlay P, Francannet C *et al.* Facial anomalies in D-2-hydroxyglutaric aciduria. *Am J Med Genet* 1999; **86**: 124.
13. Bayar A, Acun C, Dursun A *et al.* Metaphyseal enchondrodysplasia with 2-hydroxyglutaric aciduria: observation of a third case and further delineation. *Clin Dysmorphol* 2005; **14**: 7.
14. Talkhani IS, Saklatvala J, Dwyer J. D-2-hydroxyglutaric aciduria in association with spondyloenchondromatosis. *Skeletal Radiol* 2000; **29**: 289.
15. Honey EM, van Rensburg M, Knoll DP *et al.* Spondyloenchondromatosis with D-2-hydroxyglutaric aciduria; a report of a second patient with this unusual combination. *Clin Dysmorphol* 2003; **12**: 95.
16. Misra VK, Struys EA, O'Brien W *et al.* Phenotypic heterogeneity in the presentation of D-2-hydroxyglutaric aciduria in monozygotic twins. *Mol Genet Metab* 2005; **8**: 200.
17. Clarke NF, Andrews I, Carpenter K *et al.* D-2-hydroxyglutaric aciduria: a case with an intermediate phenotype and prenatal diagnosis of two affected fetuses. *Am J Hum Genet* 2003; **76**: 358.
18. Korman SH, Salomons GS, Gutman *et al.* D-2-hydroxyglutaric aciduria and glutaric aciduria type 1 in siblings: coincidence, or linked disorders? *Neuropediatrics* 2004; **35**: 151.
19. Steenweg ME, Salomons GS, Yapici Z *et al.* L-2-hydroxyglutaric aciduria: pattern of MR imaging abnormalities in 56 patients. *Radiology* 2009; **251**: 856.
20. Wang X, Jakobs C, Bawle EV. D-2-hydroxyglutaric aciduria with absence of corpus callosum and neonatal intracranial haemorrhage. *J Inherit Metab Dis* 2003; **26**: 92.
21. Baker NS, Sarnat HP, Jack RM *et al.* D-2-hydroxyglutaric aciduria: hypotonia, cortical blindness, seizures, cardiomyopathy and cylindrical spirals in skeletal muscle. *J Child Neurol* 1997; **12**: 31.

22. Gibson KM, TenBrink HJ, Schor R *et al*. Stable-isotope dilution analysis of D- and L-2-hydroxyglutaric acid: application to the detection and prenatal diagnosis of D- and L-2-hydroxyglutaric aciduria. *Pediatr Res* 1993; **34**: 277.
23. Struys EA, Jansen EEW, Verhoeven NM, Jakobs C. Measurement of urinary D- and L-2-hydroxyglutarate enantiomers by stable-isotope-dilution liquid chromatography-tandem mass spectrometry after derivatization with diacetyl-L-tartaric anhydride. *Clin Chem* 2004; **50**: 1391.
24. Rashed MS, AlAmoudi M, Aboul-Enein HY. Chiral liquid chromatography tandem mass spectrometry in the determination of the configuration of 2-hydroxyglutaric acid in urine. *Biomet Chromatogr* 2000; **14**: 317.
25. Kohlschutter A, Behbehani A, Lagenbeck U *et al*. A familial progressive neurodegenerative disease with e-oxoglutaric aciduria. *Eur J Pediatr* 1982; **138**: 32.
26. Struys EA, Verhoeven NM, Roos B, Jakobs C. Disease-related metabolites in culture medium of fibroblasts from patients with D-2-hydroxyglutaric aciduria L-2-hydroxyglutaric aciduria and combined D/L-2-hydroxyglutaric aciduria. *Clin Chem* 2003; **49**: 1133.
27. Struys EA, Verhoeven NM, Brunengraber H, Jakobs C. Investigations by mass isotopomer analysis of the formation of D-2-hydroxyglutarate by cultured lymphoblasts from two patients with D-2-hydroxyglutaric aciduria. *FEBS Lett* 2004; **12**: 29.
28. Wickenhagen WV, Salomons JGS, Gibson KM. Measurement of D-2-hydroxyglutarate dehydrogenase activity in cell homogenates derived from D-2-hydroxyglutaric aciduria patients. *J Inherit Metab Dis* 2009; **32**: 264.
29. Kaufman EE, Nelson T, Fales HM, Levine DM. Isolation and characterization of a hydroxyl-acid-oxoacid transhydrogenase from rat kidney mitochondria. *J Biol Chem* 1988; **263**: 16872.
30. Gibson KM, Sweetman L, Nyhan WL *et al*. Demonstration of 4-aminobutyric acid aminotransferase deficiency in lymphocytes and lymphoblasts. *J Inherit Metab Dis* 1985; **8**: 204.
31. Kranendijk M. Novel form of D-2-hydroxyglutaric aciduria caused by autosomal dominant gain-of-function mutations in IDH2. Late Breaking News, Annual Symposium of SSIEM, Istanbul, Turkey. *Science* 2010; **330**: 336.
32. Goodman SI, Reale M, Berlow S. Glutaric acidemia type II: a form with deleterious intrauterine effects. *J Pediatr* 1983; **102**: 411.
33. Kolker S, Pawlak V, Ahlemeyer B *et al*. NMDA receptor activation and respiratory chain complex V inhibition contribute to neurodegeneration in D-2-hydroxyglutaric aciduria. *Eur J Neurosci* 2002; **16**: 21.
34. Da Silva CG, Ribeiro CA, Leipnitz G *et al*. Inhibition of cytochrome coxidase activity in rat cerebral cortex and human skeletal muscle by D-2-hydroxyglutaric acid *in vitro*. *Biochim Biophys Acta* 2002; **1586**: 81.

L-2-hydroxyglutaric aciduria

Introduction	85	Treatment	88
Clinical abnormalities	86	References	88
Genetics and pathogenesis	87		

MAJOR PHENOTYPIC EXPRESSION

Ataxia, hypotonia, tremor, psychomotor impairment, seizures; rarely neonatal expression with apnea; cerebellar atrophy; L-2-hydroxyglutaric aciduria and deficiency of L-2-hydroxyglutarate dehydrogenase.

INTRODUCTION

L-2-hydroxyglutaric aciduria was first described by Duran and colleagues [1] in 1980 in a five-year-old Moroccan boy with psychomotor impairment. A survey of eight patients, including one by Barth in 1992 [2], and a later report by Barth and colleagues [3], established the usual phenotype of mental impairment and cerebellar signs with onset after the first year of life.

L-2-hydroxyglutaric acid (Figure 11.1) is found in increased concentrations in the urine, blood, and cerebro-

spinal fluid. The enzyme that is defective in this disease is a newly discovered, FAD-linked dehydrogenase that converts L-2-hydroxyglutarate to 2-oxoglutarate (Figure 11.1) [4]. The gene was mapped by Topcu *et al.* [5] by homozygosity mapping to 14q22.1. Mutations in the gene were found independently by these authors and by Rzem *et al.* [4]. At least 30 different mutations have been identified [6], 17 of which lead to a truncated protein. The enzyme acts as a metabolite repair enzyme [6] which catalyzes the conversion of L-2-hydroxyglutarate formed in the malate dehydrogenase reaction back to 2-oxoglutarate.

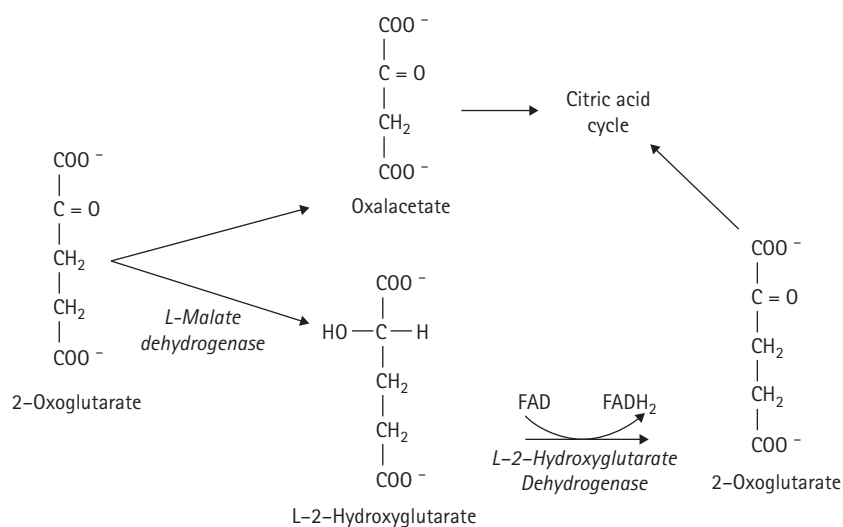


Figure 11.1 The structure of L-2 hydroxyglutaric acid, its formation and metabolite repair, thought the action of L-2-hydroxyglutarate dehydrogenase, the site of the defect in L-2-hydroxyglutaric aciduria.



Figure 11.2 A girl with L-2-hydroxyglutaric aciduria. She was slightly delayed in her early development. At two years of age, she developed grand mal seizures and progressive ataxia. By nine years of age, pyramidal signs were evident. (This figure was kindly provided by Dr Georg Hoffmann of the University of Heidelberg, Germany.)

CLINICAL ABNORMALITIES

Patients have generally appeared well for the first year [3]. Delay in walking, abnormal gait, delay in speech, and febrile seizures have been the presenting complaints in seven of 12 patients [3]. In four patients, learning disability in school first called attention to the disease. In one, cerebellar signs at ten years of age were the first evidence of disease recognized. At least 80 patients are known [6]. Imaging of the central nervous system has revealed abnormal loss of subcortical white matter and cerebellar atrophy [2, 3]. We reported [7] a patient with a much more severe phenotype who presented with disease that was rapidly fatal by 28 days of life.

Cerebellar manifestations were prominent in all but one of the patients summarized by Barth *et al.* [3]. Ataxia, dysarthria, and dysmetria were present. Impaired mental development was observed in all. Seizures were prominent in half of ten patients (Figure 11.2). They were either febrile or nonfebrile grand mal seizures. Spasticity has been observed [8].

Progressive deterioration was documented in one patient [8] after a number of years of relative stability; by 16 years, she was unable to walk and had repeated seizures. Progression was also reported in two patients by Divry and colleagues [9], who emphasized ataxia, brisk tendon reflexes, and positive Babinski as extrapyramidal

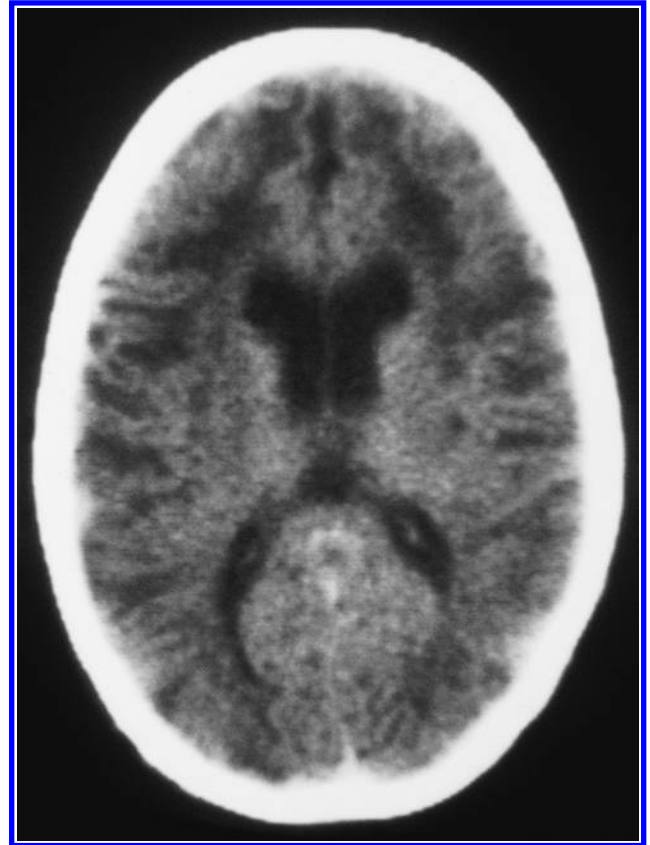


Figure 11.3 Computed tomography scan of the brain of a two-year-old with L-2-hydroxyglutaric aciduria, illustrating cerebellar atrophy and increased size of the ventricles. (This figure was kindly provided by Dr Georg Hoffmann of the University of Heidelberg, Germany.)

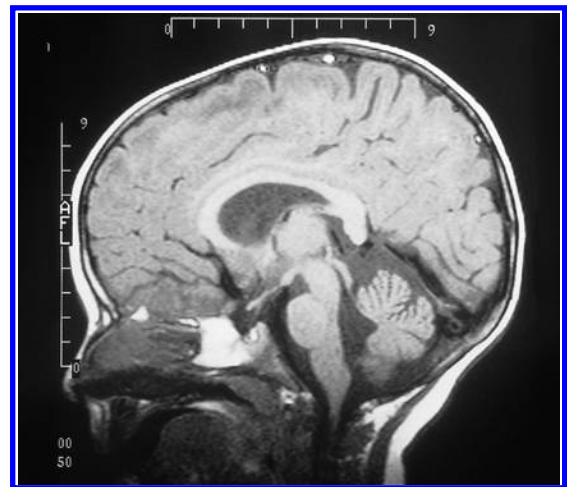


Figure 11.4 A three-year-old with L-2-hydroxyglutaric aciduria had pronounced atrophy of the cerebellum. (This figure was kindly provided by Dr Georg Hoffmann of the University of Heidelberg, Germany.)

signs and first reported macrocephaly in both patients. Macrocephaly was also observed by Wilcken *et al.* [10] in three Australian patients of Serbian, Iranian, and Iraqi parents; one demonstrated rapid neurological deterioration over five months and died; the others did not. One had strabismus and myopia. Two adult Japanese patients had seizures in childhood and psychomotor impairment, but began progressive degeneration after 25 years of age [11]. Nocturnal myoclonus was a feature. In addition to typical white matter disease on magnetic resonance imaging (MRI), the calvarium was thickened. Another 15-year-old boy [12] was wheelchair-bound and had impaired mental development epilepsy, optic atrophy, spastic tetraparesis, and dystonia. Six Iranian children, 4–16 years of age, had macrocephaly, ataxia, and progressive neurologic dysfunction [13].

A very different clinical picture was exemplified by a female patient who was limp at birth and had poor respiratory effort and bradycardia [7]. Initial pO_2 was 18, and Apgar scores at 1, 5, and 10 minutes were 3, 6, and 8, respectively. At 80 minutes, there was profound apnea and cyanosis requiring assisted ventilation. Episodic seizures began on day 2, and the electroencephalograph (EEG) revealed a burst suppression pattern and focal epileptogenic activity. Moro, grasp, and suck reflexes were absent. She died on day 28 after the withdrawal of life support.

Imaging of the central nervous system (Figures 11.3 and 11.4) in the older patients [3, 8, 13] by MRI or computed tomography (CT) scan revealed loss of substance in the subcortical white matter and cerebellar atrophy, as well as increased ventricular size. On MRI, there was decreased signal on T_1 and increased signal on T_2 in subcortical areas. The caudate nuclei were atrophic and there were signal changes in the putamen. In the cerebellum, folial atrophy involved the vermis particularly, and there were signal changes in the dentate nuclei. Magnetic resonance spectroscopy showed abnormalities in the gray, as well as white matter [8], indicating neuronal loss and neurodegeneration. The pattern on neuroimaging has been stated to be unique among neurodegenerative disorders [3]. A parasellar arachnoid cyst was observed in one patient [9]. In the infant with the rapidly fatal presentation, CT scan at 1 day of age revealed hypodense cerebellar white matter [7]. By 2 weeks, this had become more hypodense, and the cerebellum was small. In one patient, calcifications were observed in the frontal lobe [14].

A brain tumor (an ependymoma) was found in a 17-year-old boy with L-2-hydroxyglutaric aciduria, and seven other patients have been identified with brain tumors, suggesting an increased risk [15].

Neuropathology was reported in the infant who died at 28 days [7]. The brain stem and cerebellum were disproportionately small. The most striking changes were in the neocerebellum. The folia were small and illustrated patchy dropout of Purkinje cells. There was striking astrogliosis of the white matter in an olivopontocerebellar distribution. In a 15-year-old boy [15], cortical neuronal

loss was accompanied by intense gliosis and spongiosis and vacuolation in the subcortical white similar to that found in Canavan disease.

GENETICS AND PATHOGENESIS

The disease is autosomal recessive in transmission [3]. Many families have had more than one affected offspring, and males and females have been similarly affected. A number of families has been consanguineous [3, 9, 10].

Organic analysis of the urine is the usual method of detection, although analysis of the cerebrospinal fluid [15] can also serve in case finding. The concentration in the cerebrospinal fluid (CSF) was greater than that of the plasma in our patient [7]. Gas chromatography-mass spectrometry (GCMS) reveals a large quantity of 2-hydroxyglutaric acid. It is essential to determine the optical configuration of the compound identified because D-2-hydroxyglutaric aciduria (Chapter 10) is a different disease. A stable isotope dilution, internal standard, selected ion monitoring; GCMS method has been developed [14] in which the D- and L-acids are separated as the O-acetyl-di-2-butyl esters. In 13 patients, the concentrations of L-2-hydroxyglutaric acid in the urine were 1283 ± 676 mmol/mol creatinine (range, 332–2742). In control subjects, the range was 1.3–19. In some patients, the cerebrospinal fluid concentrations may be greater than that of the plasma [3] or about equal [8]. The CSF concentration was 62 ± 30 mmol/L (range, 34–100), while that of the plasma was 47 ± 13 mmol/L (range, 27–62). The control ranges were 0.3–2.3 and 0.5–10 mmol, respectively [3, 14].

Prenatal diagnosis is feasible using this method. The normal amniotic fluid concentration of L-2-hydroxyglutaric acid is 3.1–5.2 mmol/L [14].

Elevated concentrations of lysine have been observed in plasma and CSF [3, 6] or only in CSF [9, 14], but loading with L-lysine did not increase excretion of L-2-hydroxyglutaric acid. In the infant with rapidly fatal disease, concentrations of lysine were normal [16].

Abnormalities in concentrations of carnitine or dicarboxylic acids have not been found [3]. Levels of pipercolic acid were normal [3]. The increased concentration of 2-hydroxyglutaric acid in the CSF indicates endogenous origin and suggested direct involvement in pathogenesis.

The molecular nature of this disease remained elusive until quite recently [4–6]. L-2-hydroxyglutarate was not a known intermediate in any eukaryotic metabolic pathway. Archaea that produce methane utilize methopterin, a cofactor which is made from L-2-hydroxyglutarate [17]. A radiochemical assay was utilized to search for an enzyme with high affinity acting on a compound that is normally not found in mammalian tissues, and tritium was released from a racemic DL-2-hydroxy [2- 3H] glutarate. Ion exchange chromatography separated two enzymes, D-2-hydroxyglutarate dehydrogenase and L-2-hydroxyglutarate dehydrogenase. This enzyme is stimulated by FAD and active

in mitochondria of many tissues. It could not be purified. Search of databases for a FAD-linked dehydrogenase acting on L-2-hydroxyglutarate yielded a bacterial enzyme that oxidizes L-malate. Homology search using the bacterial sequence identified a human protein (C14orf160) which had a mitochondrial targeting peptide. The gene which has been named *L2HGDH* is located on chromosome 14q22.1 and has ten exons and more than 75 Kb [4]. Homozygous mutations were found in three unrelated consanguineous families. Two of them, Lys71Glu and Glu 176Asp, replaced highly conserved residues, and the third deleted an entire exon. It was concluded that this gene was *L2HGDH*.

Independently, Topcu and colleagues [5] used a genome-wide scan for alterations in five consanguineous families. They localized the gene to 5.4 Mb on chromosome 14q22.1. In a narrowed area of ten genes, they found mutations in patients with the disease. They named the gene *duranin* after the author of the first paper on the disease [1]. Two Turkish families were homozygous for a mutation in exon 7 which led to P302L. In two others, a deletion at c1115 in exon 9 indicated a premature stop. In another, a transversion in intron 7 yielded aberrant splicing of exon 7. Mutations have also been reported by Vilarinho *et al.* [18], Samuraki *et al.* [19], and Sass *et al.* [20]. Seventeen mutations led to a premature stop codon, an altered reading frame, or modified splicing that would yield a truncated protein. Fourteen missense mutations changed strictly conserved or semiconserved amino acid residues with very different size or polarity.

Incubation of lymphoblasts of patients with [$^{13}\text{C}_6$] glucose and [$^2\text{H}_5$] glutamic acid indicated that L-2-hydroxyglutarate is made from 2-oxoglutarate in mitochondria [21] in a reaction catalyzed by malate dehydrogenase. This has led to the conceptualization that L-2-hydroxyglutarate is formed only because L-malate dehydrogenase is nonspecific. The *E. coli* enzyme is even less specific. Thus L-2-hydroxyglutarate dehydrogenase catalyzes a reaction of 'metabolic repair' whose purpose is to regenerate 2-oxoglutarate. It reminds us that enzymes are not perfect catalysts, and metabolites, like DNA and some proteins, need mechanisms of repair.

TREATMENT

Information on treatment is not available.

REFERENCES

- Duran M. L-2-hydroxyglutaric aciduria: an inborn error of metabolism? *J Inherit Metab Dis* 1980; **3**: 109.
- Barth PG. L-2-hydroxyglutaric acidemia: a novel inherited neurometabolic disease. *Ann Neurol* 1992; **32**: 66.
- Barth PG, Hoffmann GF, Jaeken J *et al.* L-2-hydroxyglutaric acidemia: clinical and biochemical findings in 12 patients and preliminary report on L-2-hydroxyacid dehydrogenase. *J Inherit Metab Dis* 1993; **16**: 753.
- Rzem R, Veiga-da-Cunha M, Noel G *et al.* A gene encoding a putative FAD-dependent L-2-hydroxyglutarate dehydrogenase is mutated in L-2-hydroxyglutaric aciduria. *Proc Natl Acad Sci USA* 2004; **101**: 16849.
- Topcu M, Jobard F, Halliez S *et al.* L-2-hydroxyglutaric aciduria: identification of a mutant gene C14orf160, localized on chromosome 14q22.1 *Hum Mol Genet* 2004; **13**: 2803.
- Van Schaftingen E, Rzem R, Veiga-da-Cunha M. L-2-hydroxyglutaric aciduria, a disorder of metabolite repair. *J Inherit Metab Dis* 2009; **32**: 135.
- Chen E, Nyhan WL, Jakobs C *et al.* L-2-Hydroxyglutaric aciduria: first report of severe neurodegenerative disease and neonatal death; neuropathological correlations. *J Inherit Metab Dis* 1996; **19**: 335.
- Hanefeld F, Kruse B, Bruhn H, Frahm J. *In vivo* proton magnetic resonance spectroscopy of the brain in a patient with L-2-hydroxyglutaric acidemia. *Pediatr Res* 1994; **35**: 614.
- Divry P, Jakobs C, Vianey-Saban C *et al.* L-2-hydroxyglutaric aciduria: two further cases. *J Inherit Metab Dis* 1993; **16**: 5.
- Wilcken B, Pitt J, Health D *et al.* L-2-hydroxyglutaric aciduria: three Australian cases. *J Inherit Metab Dis* 1993; **16**: 501.
- Fujitake J, Ishikawa Y, Fujii H. L-2-hydroxyglutaric aciduria: two Japanese adult cases in one family. *J Neurol* 1999; **246**: 378.
- Seijo-Martínez M, Navarro C, Castro del Río M *et al.* L-2-hydroxyglutaric aciduria: clinical, neuroimaging, and neuropathological findings. *Arch Neurol* 2005; **62**: 666.
- Shafeghati Y, Vakili G, Entezari A. L-2-hydroxyglutaric aciduria: a report of six cases and review of the literature. *Arch Iranian Med* 2006; **9**: 165.
- Gibson KM, ten Brink HJ, Schor DS *et al.* Stable-isotope dilution analysis of D- and L-2-hydroxyglutaric acidemias. *Pediatr Res* 1993; **34**: 277.
- Aghili M, Zahedi F, Rafiee E. Hydroxyglutaric aciduria and malignant brain tumor: a case report and literature review. *J Neurooncol* 2009; **91**: 233.
- Hoffman GF, Meier-Augenstein W, Stockler S *et al.* Physiology and pathophysiology of organic acids in cerebrospinal fluid. *J Inherit Metab Dis* 1993; **16**: 648.
- Van Beelen P, Stassen AP, Bosch JW *et al.* Elucidation of the structure of methanopterin, a coenzyme from *Methanobacterium thermoautotrophicum*, using two-dimensional nuclear-magnetic-resonance techniques. *Eur J Biochem* 1984; **138**: 563.
- Vilarinho L, Cardoso ML, Gaspar P *et al.* Novel L2HGDH mutations in 21 patients with L-2-hydroxyglutaric aciduria of Portuguese origin. *Hum Mutat* 2005; **26**: 395.
- Samuraki M, Komai K, Hasegawa *et al.* A successfully treated adult patient with L-2-hydroxyglutaric aciduria. *Neurology* 2008; **70**: 1051.
- Sass JO, Jobard F, Topcu M *et al.* L-2-hydroxyglutaric aciduria: identification of ten novel mutations in the L2HGDH gene. *J Inherit Metab Dis* 2008; **3**: 109.
- Struys EA, Gibson KM, Jakobs C. Novel insights into L-2-hydroxyglutaric aciduria: mass isotopomer studies reveal 2-oxoglutaric acid as the metabolic precursor of L-2-hydroxyglutaric acid. *J Inherit Metab Dis* 2007; **30**: 690.

4-Hydroxybutyric aciduria

Introduction	89	Treatment	93
Clinical abnormalities	89	References	93
Genetics and pathogenesis	92		

MAJOR PHENOTYPIC EXPRESSION

Mental impairment, ataxia, hypotonia, hyporeflexia, convulsions, hyperkinetic behavior, or lethargy bordering on narcolepsy, macrocephaly, excretion of 4-hydroxybutyric acid in the urine, and deficiency of succinic semialdehyde dehydrogenase.

INTRODUCTION

4-Hydroxybutyric aciduria [1, 2] is a metabolic disorder that serves as a model for conditions in which the metabolic block causes the accumulation of a compound of established neuropharmacologic activity. Actually, since 4-aminobutyric acid (GABA) also accumulates in this disease, the disease is unique in the increased concentrations of two neuroactive compounds.

4-Hydroxybutyric acid was once developed as an intravenous anesthetic in order to obtain an analog of GABA, which would cross the blood–brain barrier. However, on testing in animals, it was found to produce convulsions [3–5] and thus it never came to human trials. The first patient with 4-hydroxybutyric aciduria was described by Jakobs and colleagues [1] in 1981. Thirty-one patients in 21 families were studied by 1983 [2, 6, 7]. A report [8] of experience with 23 patients emphasized the importance and difficulty of organic acid analysis in the diagnosis of this disorder.

Among disorders of GABA metabolism 4-hydroxybutyric aciduria has been more frequently encountered, probably because the key intermediate 4-hydroxybutyric acid is detectable by analysis of organic acids [2]. The fundamental defect is in the activity of the succinic semialdehyde dehydrogenase (EC 1.2.1.24) (Figure 12.1). In the reaction catalyzed by this enzyme, the product of GABA transamination is normally converted to succinic acid and hence to oxidation via the citric acid cycle [9]. 4-Hydroxybutyric acid is converted via β -oxidation into

3,4-dihydroxybutyric acid and thereafter to its keto acid, to glycolaldehyde and glycolic acid [10].

The genes for rat and human semialdehyde dehydrogenase have been cloned [11, 12]. The locus for the human gene (*ALDH5A1*) is chromosome 6p22 [12, 13]. Mutation analysis elucidated two exon-skipping mutations at consensus splice sites in four patients in two families [13]. A mutational spectrum has been observed in more than 50 patients from many unrelated families worldwide [14].

CLINICAL ABNORMALITIES

Impaired psychomotor development is a common feature of patients with 4-hydroxybutyric aciduria and may be severe (Figures 12.2 and 12.3). Most have had delayed development of speech [15]. The first patient presented at 20 months of age with impaired motor development (Figure 12.4). He could not stand, walk, or speak. He had had brief convulsions between six and 12 months. He was ataxic and hypotonic, but not weak. The electroencephalograph (EEG) was diffusely abnormal, and computed tomography (CT) scan revealed cerebral atrophy. Bone age was impaired. At five years of age, his condition was described as stable, without deterioration [16]. He still had no speech and an ataxic gait.

Nonprogressive ataxia and hypotonia have been recognized as characteristic of this syndrome [2, 15–20], along with relatively mild mental impairment. Two siblings, first seen at nine and 11 years of age, had moderate ataxia

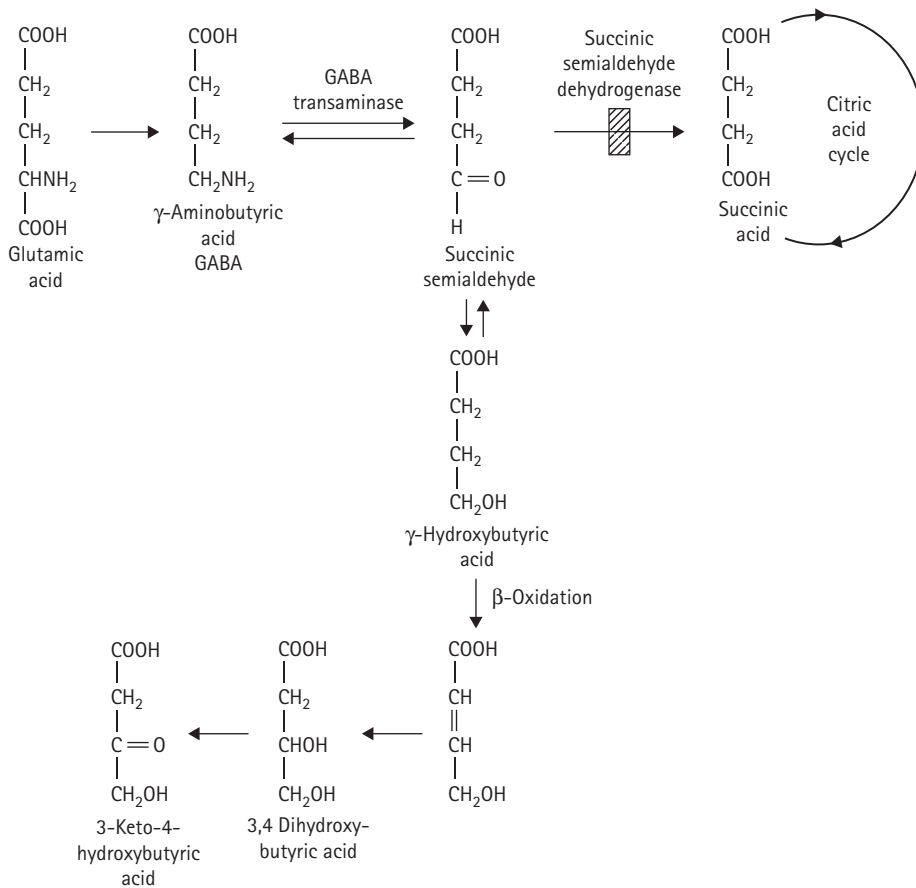


Figure 12.1 Succinic semialdehyde dehydrogenase, the site of defect in 4-hydroxybutyric aciduria, and the formation and metabolism of 4-hydroxybutyric acid.



Figure 12.2 BR: A four-year-old boy with 4-hydroxybutyric aciduria. He was mentally impaired and was later admitted to an institution. (Illustration was kindly provided by Dr Priscille Divry, Hopital Debrousse, Lyon, France.)



Figure 12.3 Close-up view of the patient in Figure 12.2. (Illustration kindly provided by Dr Priscille Divry, Hopital Debrousse, Lyon, France.)



Figure 12.4 SB: The index patient with 4-hydroxybutyric aciduria (Case report). (Illustration kindly provided by Dr Dietz Rating, now of the University of Heidelberg, Germany.)

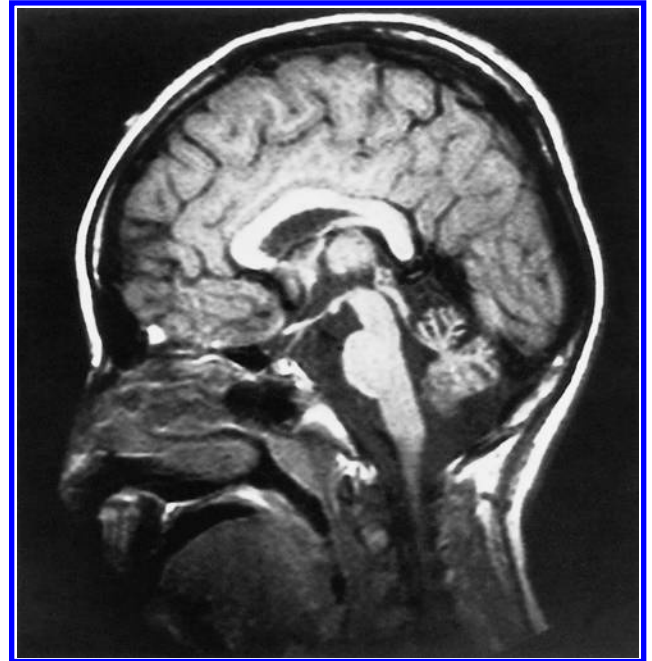


Figure 12.6 Magnetic resonance scan of the brain of a patient with 4-hydroxybutyric aciduria, illustrating cerebral atrophy.



Figure 12.5 RF and RM: Lebanese siblings with 4-hydroxybutyric aciduria. (Illustration was kindly provided by Dr Deitz Rating, Heidelberg, Germany.)

and intention tremor (Figure 12.5). Their speech was mildly dysarthric. The girl was hypotonic, but her brother was not. Deep tendon reflexes were difficult to elicit. Two years later, the ataxia in both of these children had improved considerably. Another patient had mental impairment and hypertonia and ataxic, and no improvement was noted with time. At six years of age, he could hardly stand and could not walk. Seizures began before one year of age. Sensory examination was normal in all of the children. Seizures occurred in slightly less than half of a group of reported patients [15]. Hypotonia was observed in 74 percent. Optokinetic nystagmus has been described [15].



Figure 12.7 A nine-year-old girl with 4-hydroxybutyric aciduria. Her extreme hyperkinetic behavior had led to treatment with thioridazine, which ultimately led to the dyskinesia illustrated.

Both microcephaly and macrocephaly have been observed. One patient underwent a pyloromyotomy [15].

The clinical spectrum of the disease was expanded [8] in an assessment of 51 reported patients and a new cohort of patients with deficiency of the dehydrogenase. Mental

impairment, disproportionate dysfunctioning language, hypotonia, and seizures were uniformly encountered. Only one patient presented with acute encephalopathy; she also had elevated levels of glycine [21]. Magnetic resonance imaging (MRI) of the brain may be normal [17] or may reveal cerebral atrophy (Figure 12.6). One patient had a normal MRI, followed by symmetric lesions in the globus pallidus, thalamus, and brain stem four years later [15]. Increased T_2 -weighted symmetrical signal in the globus pallidus may be the more characteristic finding [8]. Seizures may be generalized tonic-clonic or myoclonic. EEG findings have included generalized spike and wave discharges in sleep, temporal focal spikes, and background slowing. Hallucinations have been reported [8].

Extremes of activity have been observed in different patients or families. Extremely hyperkinetic behavior has been the characteristic mode for some patients (Figure 12.7) [17, 18]. Others, such as a pair of siblings we have studied, were lethargic and somnolent to a degree that suggested narcolepsy. Some have been thought to be autistic [8, 18]. Ataxia has been observed to resolve with age [22].

A possibility of two groups of phenotypes was suggested [8] by some patients whose early development was normal. Even so, ultimate disease was not mild.

GENETICS AND PATHOGENESIS

4-Hydroxybutyric aciduria is an autosomal recessive disorder. In seven of 21 families of probands, the parents were consanguineous [1, 2, 6, 16]. Intermediate levels of enzyme activity have been found in parents [19, 20].

Prenatal diagnosis of an affected fetus has been accomplished [23] by gas chromatography-mass spectrometry (GCMS) assay of the concentration of 4-hydroxybutyric acid in amniotic fluid. Enzyme activity was absent in fetal brain, liver, and kidney [24]. Succinic semialdehyde dehydrogenase is also measurable in chorionic villus samples [25], providing another avenue for prenatal diagnosis.

The molecular defect in 4-hydroxybutyric aciduria is in the enzyme succinic semialdehyde hydrogenase (EC 1.2.1.2) (Figure 12.1) [7, 20, 25, 26]. Succinic semialdehyde is the product of the transamination of GABA and is normally converted to succinic acid. When succinic semialdehyde accumulates, it is reduced to 4-hydroxybutyric acid. The enzyme is active in lymphocytes freshly isolated from peripheral blood and in cultured lymphoblasts [7, 19, 25, 26]. Accumulation of labeled succinic semialdehyde has been demonstrated in patients' lymphocytes following incubation with ^{14}C -labeled GABA, and there was no evidence of further metabolism to succinic acid [7]. Direct assay of the enzyme with ^{14}C -labeled succinic semialdehyde yielded activity that approximated 4 percent of the control level in one patient [25] and was undetectable in another [20]. In another [18], it was as high as 21 percent in lymphocytes.

Although clinical expression has been highly variable with quite mild and severe or even fatal disease, the degree of phenotypic variation has not correlated with the amount of residual enzyme activity [2] even when monitored by a whole cell assay in which levels of activity tend to be higher [27, 28].

The cDNA for succinic acid semialdehyde dehydrogenase has been isolated from *E. coli* [29, 30]. Characterization of the human gene was accomplished following purification of the mammalian enzyme. Studies of the nature of mutation have begun [13, 31]. Three splicing errors have led to losses of exon 8, 9, and part of 4. In addition, an insertion, a deletion, four nonsense mutations, and a number of missense mutations have been reported [31]. The most common mutation (W204X) appears to be a founder mutation among Europeans [14]. The second most common (R412X) was found in various locations. Among a spectrum of mutations [14], most missense mutations have led to an enzymatic phenotype of less than 5 percent of control activity in an *in vitro* expression system. Thus residual activity is not likely to account for the very large differences in clinical phenotype.

The immediate consequence of the metabolic block is the accumulation of 4-hydroxybutyric acid. This compound has been found in large amounts in the urine in all of the studied patients [1, 2, 16, 32]. Its nature was documented by GCMS. In the index patient [1], the amounts excreted varied from 170 to 340 mmol/mol creatinine. Concentrations in urine have ranged from two- to 500-fold the normal level [33, 34]. Succinic semialdehyde may be found in the urine, but the amounts are small. They ranged from 5 to 10 mmol/mol creatinine in one patient [1]. The ratio of 4-hydroxybutyric acid to succinic semialdehyde approximated 35 times. 3,4-Dihydroxybutyric acid has also been found regularly in the urine, and what appeared to be its 3-keto analog.

Increased concentrations of 4-hydroxybutyric acid are also found in the cerebrospinal fluid (CSF) [1, 2, 6, 32] and in the plasma. In the index patient, the concentration in the cerebrospinal fluid was 600 mm/L, approximately 60 percent of the levels found in the plasma [1], and in the second patient it was 350 mm/L, almost three times that of the plasma [6]. Overall elevations have ranged from 100- to 1200-fold [33, 34]. 4-Hydroxybutyric acid is not readily detected in the CSF of control individuals. Quantification of this compound, even in patients, may be spuriously low. A stable isotope dilution, internal standard method [33] has revealed consistently higher levels and would be best for prenatal diagnosis. It has been emphasized [8] that patients may be missed in the usual methods of GCMS analysis for organic acids. The use of selective ion monitoring mass spectrometry has resulted in the diagnosis of increased numbers of patients. Administration of glutamic acid increased the plasma concentration of 4-hydroxybutyric acid [6]. GABA has also been found in increased concentration in the CSF. The level of 654 pm/mL [1] was over six times the control mean.

Among patients with 4-hydroxybutyric aciduria, concentrations have been higher in younger and lower in older patients [33, 34]. This could reflect changing ratios of brain mass to body mass with age. It could provide an explanation for somnolence in young patients and hyperactivity or aggression in older patients. It has been suggested that 4-hydroxybutyric acid might bind to inhibitory sites at high concentrations and to excitatory sites at low concentrations [2].

Other compounds found in the urine of patients include dicarboxylic acids, which might suggest a disorder of fatty acid oxidation [35, 36]. 4-Hydroxybutyric acid is, after all, a short chain fatty acid. They could result from secondary inhibition of mitochondrial fatty acid oxidation. 4, 5-Dihydroxyhexanoic acid identified in the urine of these patients [35] has not been found in other metabolic diseases and so may be a specific marker for this disease. It could arise from the condensation of a 2-carbon moiety with succinic semialdehyde. The occurrence of 3-hydroxypropionic acid and glycine in the urine of some patients might suggest a diagnosis of a disorder of propionate metabolism. Identification of the key compound, 4-hydroxybutyric acid, should avoid any confusion. Glycine would be a product of glycolic acid, which can be formed from β -oxidation of 4-hydroxybutyric acid [37].

TREATMENT

Treatment has been undertaken with vigabatrin (γ -vinyl-GABA), which is an irreversible inhibitor of GABA transaminase [2, 18, 38, 39]. Cerebellar signs were reported in five of six patients treated [2]. Doses employed have included 1.5 g/day in a 30-kg patient [18] in whom alertness appeared to improve and hypotonia to decrease. Long-term efficacy remains to be established. At least one patient was reported not to improve. Patients should be closely monitored because the drug may be expected to increase levels of GABA in the central nervous system and, as indicated by GABA transaminase deficiency, this would be expected to cause neurologic disease. Several treated patients have developed seizures [40]. Overall results have been inconsistent [8]. Valproate is an inhibitor of succinic semialdehyde dehydrogenase and it is contraindicated. Methylphenidate may decrease daytime somnolence [41].

REFERENCES

- Jakobs C, Bojasch M, Monch E *et al*. Urinary excretion of gamma-hydroxybutyric acid in a patient with neurological abnormalities. The probability of a new inborn error of metabolism. *Clin Chim Acta* 1981; **111**: 169.
- Jakobs C, Jaeken J, Gibson KM. Inherited disorders of GABA metabolism. *J Inherit Metab Dis* 1993; **16**: 704.
- Labroit H, Jouany J, Gerard J, Fabiani F. Report of a clinical and experimental study. Sodium 4-hydroxybutyrate a metabolite substrate with central inhibitory activity. *Presse Med* 1960; **68**: 1867.
- Godschalk M, Dzoljic MRI, Bonta IL. Slow wave sleep and a state resembling absence epilepsy induced in the rat by gamma-hydroxybutyrate. *Eur J Pharmacol* 1977; **44**: 105.
- Snead OC. Gamma-hydroxy-butyrate in the monkey I. Electroencephalographic behavioral and pharmacokinetic studies. *Neurology* 1978; **28**: 638.
- Divry P, Baltassat P, Rolland MO *et al*. A new patient with 4-hydroxybutyric aciduria a possible defect of 4-aminobutyrate metabolism. *Clin Chim Acta* 1983; **129**: 303.
- Gibson KM, Sweetman L, Nyhan WL *et al*. Succinic semialdehyde dehydrogenase deficiency: an inborn error of gamma-aminobutyric acid metabolism. *Clin Chim Acta* 1983; **133**: 33.
- Pearl PL, Gibson KM, Acosta MT *et al*. Clinical spectrum of succinic semialdehyde dehydrogenase deficiency. *Neurology* 2003; **5**: 1413.
- Roberts E (ed.). *Inhibition in the Nervous System and γ -Aminobutyric Acid*. New York: Pergamon Press, 1960.
- Walkenstein SS, Wiser R, Gudmundsen C, Kimmel H. Metabolism of 4-hydroxybutyric acid. *Biochim Biophys Acta* 1964; **86**: 640.
- Chambliss KL, Claudle DL, Hinson DD *et al*. Molecular cloning of the mature NAD(1)-dependent succinic semialdehyde dehydrogenase from rat and human: cDNA isolation evolutionary homology and tissue expression. *J Biol Chem* 1995; **270**: 461.
- Trettel F, Malaspina P, Jodice C *et al*. Human succinic semialdehyde dehydrogenase: molecular cloning and chromosomal localization. *Adv Exp Med Biol* 1997; **414**: 253.
- Chambliss KL, Hinson DD, Trette F *et al*. Two exon skipping mutations as the molecular basis of succinic semialdehyde dehydrogenase deficiency (4-hydroxybutyric aciduria). *Am J Hum Genet* 1998; **63**: 399.
- Akaboshi S, Hogema BM, Novelletto A *et al*. Mutational spectrum of the succinate semialdehyde dehydrogenase (ALDH5A1) gene and functional analysis of 27 novel disease-causing mutations in patients with SSADH deficiency. *Hum Mutat* 2003; **22**: 442.
- Gibson KM, Christensen E, Jakobs C *et al*. The clinical phenotype of succinic semialdehyde dehydrogenase deficiency (4-hydroxybutyric aciduria): case reports of 23 new patients. *Pediatrics* 1997; **99**: 567.
- Rating D, Hanefeld F, Siemes H *et al*. 4-hydroxybutyric aciduria: a new inborn error of metabolism I. Clinical review. *J Inherit Metab Dis* 1984; **7**: 92.
- Gibson KM, Hoffmann G, Nyhan WL *et al*. 4-Hydroxybutyric aciduria in a patient without ataxia or convulsions. *Eur J Pediatr* 1988; **147**: 529.
- Uziel G, Bardelli P, Pantaleoni C *et al*. 4-Hydroxybutyric aciduria: clinical findings and Vigabatrin therapy. *J Inherit Metab Dis* 1993; **16**: 520.
- Gibson KM, Sweetman L, Nyhan WL *et al*. Demonstration of 4-aminobutyric acid aminotransferase deficiency in

- lymphocytes and lymphoblasts. *J Inherit Metab Dis* 1985; **8**: 204.
20. Gibson KM, Sweetman L, Jansen I *et al*. Properties of succinic semialdehyde dehydrogenase in cultured human lymphoblasts. *J Neurogenet* 1985; **2**: 111.
 21. Gibson KM, Goodman SI, Frerman FE *et al*. Succinic semialdehyde dehydrogenase deficiency associated with combined 4-hydroxybutyric and dicarboxylic acidurias: potential for clinical misdiagnosis based on urinary organic acid profiling. *J Pediatr* 1989; **114**: 607.
 22. Hodson AK, Gibson KM, Jakobs C. Developmental resolution of ataxia in succinic semialdehyde dehydrogenase deficiency. *Ann Neurol* 1990; **28**: 438.
 23. Jakobs C, Ogier H, Rabier D, Gibson KM. Prenatal detection of succinic semialdehyde dehydrogenase deficiency (4-hydroxybutyric aciduria). *Prenat Diagn* 1993; **13**: 150.
 24. Chambliss KL, Lee CF, Ogier H *et al*. Enzymatic and immunologic demonstration of normal and defective succinic semialdehyde dehydrogenase activity in fetal brain liver and kidney. *J Inherit Metab Dis* 1993; **16**: 523.
 25. Sweetman FR, Gibson KM, Sweetman L *et al*. Activity of biotin-dependent and GABA metabolizing enzymes in chorionic villus samples: potential for 1st trimester prenatal diagnosis. *Prenat Diagn* 1986; **6**: 187.
 26. Gibson KM, Nyhan WL, Jaeken J. Inborn errors of GABA metabolism. *BioEssays* 1986; **4**: 24.
 27. Pattarelli PP, Nyhan WL, Gibson KM. Oxidation of [U-¹⁴C] succinic semialdehyde in cultured human lymphoblasts: measurement of residual succinic semialdehyde dehydrogenase activity in 11 patients with 4-hydroxybutyric aciduria. *Pediatr Res* 1988; **24**: 455.
 28. Gibson KM, Lee CF, Chambliss KL *et al*. 4-Hydroxybutyric aciduria: application of a fluorometric assay to the determination of succinic semialdehyde dehydrogenase activity in extracts of cultured human lymphoblasts. *Clin Chim Acta* 1991; **196**: 219.
 29. Marek LE, Henson JM. Cloning and expression of the *Escherichia coli* K-12 GAD gene. *J Bacteriol* 1988; **170**: 991.
 30. Bartsch K, von Johnn-Marteville A, Schulz A. Molecular analysis of two genes of the *Escherichia coli* gab cluster: nucleotide sequence of the glutamate:succinic semialdehyde transaminase gene (gabT) and characterization of the succinic semialdehyde dehydrogenase gene (gabD). *J Bacteriol* 1990; **172**: 7035.
 31. Hogema BM, Jakobs C, Oudejans CBM *et al*. Mutation analysis in succinic semialdehyde dehydrogenase (SSADH) deficiency (4-hydroxybutyric aciduria). *Am J Hum Genet* 1999; **65**: A238.
 32. Jakobs C, Kneer J, Rating D *et al*. A new inborn error of metabolism: gamma-hydroxybutyric aciduria – biochemical findings. *J Inherit Metab Dis* 1984; **7**: 92.
 33. Gibson KM, Aramaki S, Sweetman L *et al*. Stable isotope dilution analysis of 4-hydroxybutyric acid: an accurate method for quantification in physiological fluids and the prenatal diagnosis of 4-hydroxybutyric aciduria. *Biomed Environ Mass Spectrom* 1990; **19**: 89.
 34. Jakobs C, Smit LME, Kneer J *et al*. The first adult case with 4-hydroxybutyric aciduria. *J Inherit Metab Dis* 1990; **13**: 341.
 35. Brown GK, Cromby CH, Manning NJ, Pollitt RJ. Urinary organic acids in succinic semialdehyde dehydrogenase deficiency: evidence of α -oxidation of 4-hydroxybutyric acid interaction of succinic semialdehyde with pyruvate dehydrogenase and possible secondary inhibition of mitochondrial β -oxidation. *J Inherit Metab Dis* 1987; **10**: 367.
 36. Ishiguro Y, Kajita M, Aoshiima T *et al*. The first case of 4-hydroxybutyric aciduria in Japan. *Brain Dev* 2001; **23**: 128.
 37. Vamecq J, Draye J-P, Poupaert JH. Studies on the metabolism of glycolyl-CoA. *Biochem Cell Biol* 1990; **68**: 846.
 38. Jakobs C, Michael T, Jaeger E *et al*. Further evaluation of Vigabatrin therapy in 4-hydroxybutyric aciduria. *Eur J Pediatr* 1992; **151**: 466.
 39. Howells D, Jakobs C, Kok RM *et al*. Vigabatrin therapy in succinic semialdehyde dehydrogenase deficiency. *Mol Neuropharmacol* 1992; **2**: 181.
 40. Gibson KM, Hoffmann GF, Hodson AK *et al*. 4-Hydroxybutyric acid and the clinical phenotype of succinic semialdehyde dehydrogenase deficiency an inborn error of GABA metabolism. *Neuropediatrics* 1998; **29**: 14.
 41. Daly DM, Hodson A, Gibson KM. Central auditory processing in a patient with SSADH deficiency. *Soc Neurosci* 1991; **17**(Part I): 892 (Abstr.).

Mitochondrial acetoacetyl-CoA thiolase (3-oxothiolase) deficiency

Introduction	95	Treatment	100
Clinical abnormalities	96	Differential diagnosis: related disorders of ketolysis	100
Genetics and pathogenesis	98	References	100

MAJOR PHENOTYPIC EXPRESSION

Acute episodes of ketosis and acidosis, vomiting, lethargy, urinary excretion of 2-methyl-3-hydroxybutyric acid, tiglylglycine and 2-methylacetoacetic acid, deficiency of mitochondrial acetoacetyl CoA thiolase (2-methylacetoacetic acid 3-oxothiolase).

INTRODUCTION

The disease was first reported in 1971 [1] as a disorder of isoleucine metabolism because of the excretion of large amounts of 2-methyl-3-hydroxybutyric and 2-methylacetoacetic acids in the urine and their increase in response to the administration of protein or isoleucine [2, 3]. Since then, more than 30 patients have been reported [4].

These patients have been characterized by the occurrence of multiple episodes of massive ketosis. They have been differentiated as defects in ketolysis [5] from

ketoacidotic disorders such as propionic acidemia, methylmalonic acidemia, and isovaleric acidemia, where the mechanism appears to be excessive production of acetoacetate. There could be elements of overproduction in this disease too, as the block (Figure 13.1) is just prior to the formation of propionyl CoA, and CoA-containing intermediates accumulate behind the block as far as tiglyl CoA. On the other hand, a major peripheral role for the mitochondrial acetoacetyl CoA thiolase, which is deficient in this disease, is in the utilization of acetoacetyl CoA and its conversion to acetyl CoA (Figure 13.2).

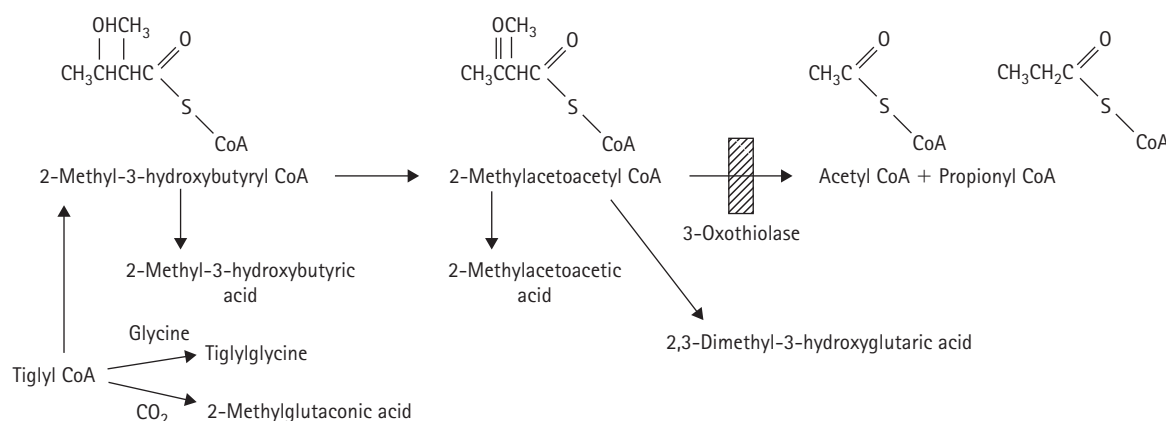


Figure 13.1 3-Oxothiolase and related reactions. In the presence of deficiency the major products of this metabolic pathway are tiglylglycine and 2-methyl-3-hydroxybutyric acid.

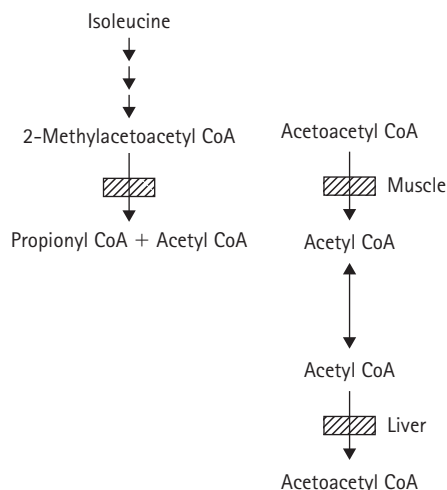


Figure 13.2 The multiple roles of mitochondrial acetyl CoA thiolase. In addition to its place in the catabolism of isoleucine, it is involved in ketone body synthesis in liver and in its utilization in peripheral tissues.

The molecular defect is in the mitochondrial short chain-length-specific thiolase, 2-methylacetoacetyl CoA thiolase (EC 2.3.1.9) [6–8]. The products of this enzymatic cleavage are acetyl CoA and propionyl CoA (Figure 13.1). The cDNA has been cloned [9] and the gene now referred to as acetylCoA acetyltransferase-1 (*ACAT1*) is located on chromosome 11 at q22.3-23.1 [10]. A number of mutations has been defined [4, 11–13].

CLINICAL ABNORMALITIES

Among the patients reported [1, 2, 8, 14–27], there has been considerable heterogeneity, but a unifying feature is the occurrence of episodes of acute illness in which there is massive ketosis and acidosis. Episodes may be ushered in by vomiting. At least one patient [18] underwent pyloromyotomy. There may be associated lethargy, coma, hyperventilation, and dehydration. This is a life-threatening illness and death has been reported [2, 4, 17]. Many have had siblings who died early in life. A few patients have had neonatal onsets, but most have presented first in late infancy or childhood. Twenty-one of 26 patients presented before 25 months of age [27].

Episodes of acute illness are induced most commonly by intercurrent infection or other cause of catabolism such as appendectomy [16]. They can also be induced by the intake of protein. During episodes of acute illness, patients often require admission to hospital and parenteral fluid therapy containing alkali. During acidosis, concentrations of sugar in the blood may be elevated. Hyperglycemia and ketoacidosis may lead to a diagnosis of diabetes mellitus and the administration of insulin. Another problem of differential diagnosis was raised by experience with a

one-year-old girl who presented with vomiting, ketosis, and severe acidosis [17] and was initially thought to have salicylate intoxication. This impression was heightened by the colorimetric test of the blood for salicylates, which gave an abnormally high reading because of cross-reaction with acetoacetic acid. Fortunately, organic acid analysis was also carried out, providing the diagnosis. Blood ammonia and lactate are usually not elevated. Levels of carnitine are low [11].

Most patients have no other clinical manifestations besides the episodic ketoacidosis. Specifically, intellectual development may be normal [1, 2, 4, 6, 16, 19]. In fact, one patient had been asymptomatic at report at 36 years [6]; at least three have not experienced ketoacidotic episodes. Congestive cardiac myopathy has been reported [18]. This might be a consequence of carnitine deficiency. Seizures have been observed, especially in the acute episode. Another patient had abdominal pain during the acute episode [8]. Some have developed mental impairment (Figure 13.3) or speech problems [2, 14, 15, 19].

In four reported patients, all had significant neurological abnormality, and in each, development was slow prior to the initial acidotic episode (Figures 13.4 and 13.5) [26]. All had severe degrees of central hypotonia. Some patients



Figure 13.3 MM: A seven-year-old Spanish girl with 3-oxothiolase deficiency. She had many episodes of severe ketoacidosis and was developmentally delayed. Gait was spastic on the right side and Babinski response was positive on the right.



Figure 13.4 MAS: A nine-month-old infant with 3-oxothiolase deficiency [26]. Hypotonia was impressive and motor development delayed.



Figure 13.5 MMS: The seven-year-old sister of MAS. She had marked impairment of motor skills.

were ataxic [4, 16, 22]. Others had severe headaches. Magnetic resonance imaging (MRI) revealed high intensity T2 lesions in the posterior lateral putamina (Figures 13.6 and 13.7). This appearance is unusual enough to suggest the diagnosis [26].

Some patients have been reported as mild examples

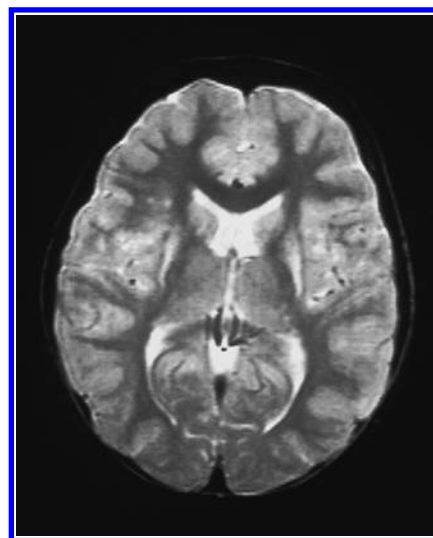


Figure 13.6 MMS: Magnetic resonance scan revealed bilateral high intensity T2 lesions in the lentiform nuclei. The nuclei were reduced in volume bilaterally.

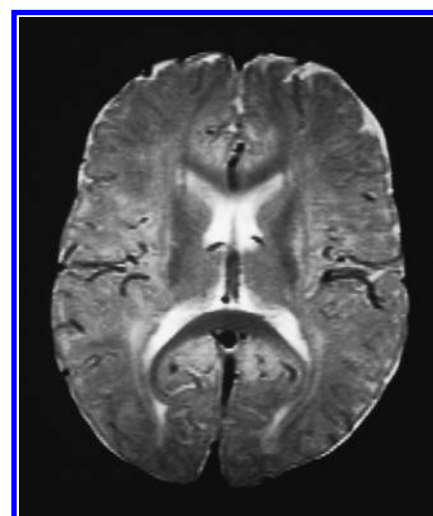


Figure 13.7 MAS: Magnetic resonance scan revealed lesions in the white matter of the external capsule.

of the disease [28, 29]. Actually, a majority of patients have done well once diagnosed. The frequency of attacks diminishes by age. In an assembly of experience with patients worldwide [4], the latest attack was at ten years of age. In addition to the three patients with no episodes, 11 had only one. Only 12 of 26 patients had recurrent episodes of ketoacidosis. A patient diagnosed at eight years of age was reported after she became pregnant at 25 years of age [30]. She appeared normal, except for the characteristic organic aciduria. She was treated with carnitine. Her infant was normal.

Concentrations of amino acids are usually normal, but three patients have had hyperglycinuria [14–16]. This is reminiscent of propionic acidemia, as were the

neutropenia, thrombocytopenia, and hyperglycinemia in one of them, a 12-week-old girl [14], who developed episodes of lethargy and vomiting. The increased quantities of 3-hydroxybutyric acid in this disease have been reported to induce fetal hemoglobin and may ameliorate a coexisting β -thalassemia. A 21-month-old patient who presented with severe metabolic acidosis had hypoglycemia and hyperuricemia [31].

The diagnostic feature of this disorder is the organic aciduria. 2-Methyl-3-hydroxybutyric acid and 2-methylacetoacetic acid and tiglylglycine are the key metabolites. They are found regularly in the urine of these patients and occur in only trace amounts in normal urine. In general, the amounts of 2-methyl-3-hydroxybutyric acid are considerably greater than those of 2-methylacetoacetic acid; the latter may even be undetectable. 2-Methyl-3-hydroxybutyric acid may be found in the urine in concentrations of 200–1000 mmol/mol of creatinine under normal circumstances, increasing at times of acute illness and in response to the administration of protein or of isoleucine to as high as 14,400 mmol/mol creatinine [2, 16, 19]. Normal individuals excrete less than 10 mmol/mol creatinine. Tiglylglycine is excreted in amounts up to 7000 mmol/mol creatinine [6, 19, 32], but some patients do not normally excrete tiglylglycine. In one of our patients [19], none of these metabolites were present in urine assayed in the absence of acute ketosis.

Organic acid analysis during ketosis can, however, be confusing. Key metabolites can be masked at the time of the acute ketosis. In addition, severe ketosis may lead in anyone to the excretion of 3-hydroxy acids, including 2-methyl-3-hydroxybutyric acid in amounts as high as 200 mmol/mol creatinine, as well as 3-hydroxyisovaleric acid [33]. During ketosis, we have also observed 2-methyl-3-hydroxybutyric acid and 2-methylacetoacetic acid in the urine of patients with propionic academia [34]. Therefore, it is important in the assessment of any nondiabetic patient who develops acute acidosis and massive ketosis to carry out analysis of the organic acids of the urine after successful treatment of the ketosis, as well as at the time of the acute illness. Patients with propionic academia can be clearly distinguished by the excretion of 3-hydroxypropionic acid and methylcitric acid. Some patients with 3-oxothiolase deficiency may not be distinguishable from anyone else with ketosis until the ketosis has subsided. Among the organic acidemias, this

is the disorder most likely to be missed by organic acid analysis. It may be necessary to administer an isoleucine load in the absence of ketosis in order to clarify the organic aciduria of this condition. We have employed a single dose of 100 mg/kg of isoleucine for this purpose. Others [2, 6, 15, 16] have given three doses of 75 mg/kg per day for 2 days. In 8 hours following 100 mg/kg, our patients have excreted up to 1000–4000 mmol/mol creatinine of 2-methyl-3-hydroxyisobutyric acid or tiglylglycine [35].

In addition to these metabolites, butanone may also be found in the urine in this disorder [2, 15, 16], and (E)-2-methylglutaconic acid appears also to be a characteristic metabolite [36]. It is thought to result from carboxylation of tiglylCoA catalyzed by 3-methylcrotonylCoA carboxylase. 2,3-Dimethyl-3-hydroxyglutaric acid has also been identified in the urine of a patient with this disease [3]. It is thought that this compound results from accumulated 2-methylacetoacetylCoA in the reaction catalyzed by 3-methyl-3-hydroxyglutarylCoA synthase, a similar reaction in propionic acidemia with propionylCoA as substrate instead of the usual acetylCoA yields 3-ethyl-3-hydroxyglutaric acid [3]. Adipic acid and other dicarboxylic acids may be found during ketosis. A characteristic acylcarnitine profile has also been observed via tandem mass spectroscopy (MS/MS) (Figure 13.8) [37]. The MS/MS diagnosis is made on the basis of tiglylcarnitine and 2-methyl-3-hydroxybutyrylcarnitine in the blood.

GENETICS AND PATHOGENESIS

The disorder is autosomal recessive (Figure 13.9). Consanguinity has been reported [2, 26], but it has been rare among families reported [4]. The disease appears to be relatively frequent in Tunisia [38]. A method for the analysis of 2-methyl-3-hydroxybutyric acid in amniotic fluid [39] should be useful in the rapid prenatal diagnosis of the disorder. Prenatal diagnosis should also be possible by assay of the enzyme in cultured amniocytes. Heterozygosity has been determined by assay of the enzyme in cultured fibroblasts [6, 40], but it may not be reliable in distinguishing an individual heterozygote from normal. In one family, the mother and brother of the patient were heterozygotes, but the father was found on enzyme assay to be a homozygote [4, 6, 11].

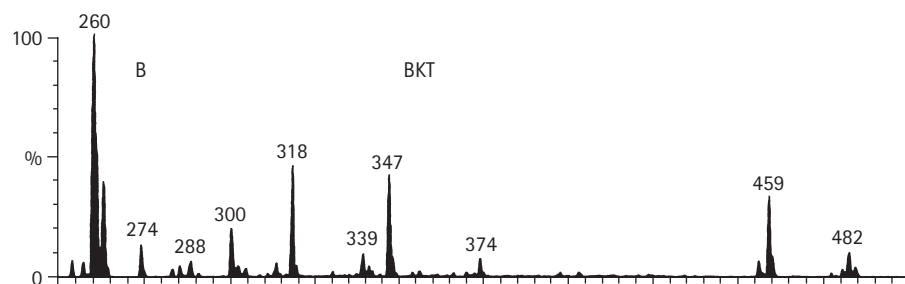


Figure 13.8 Tandem mass spectrometric profiles of acyl carnitine esters in the blood of a patient with 3-oxothiolase deficiency (BKT). The key compounds were tiglylcarnitine m/z 300 and 2-methyl-3-hydroxybutyrylcarnitine (m/z 318). (Reprinted with permission from *Pediatr Res* [37].)

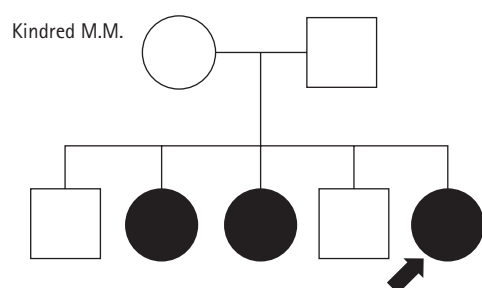


Figure 13.9 The family of the patient shown in Figure 13.3. The two previously affected siblings had died at 13 and 21 months.

The enzymatic defect in the mitochondrial acetoacetyl CoA thiolase can be demonstrated by assay of the oxidation of ^{14}C -isoleucine by cultured fibroblasts [3, 18, 41–43], or by the conversion of tritiated tiglylCoA into propionic acid [3]. Deficiency of the enzyme has also been reported in leukocytes [44]. Assay of the activity of the K^+ -dependent acetoacetyl CoA-3-ketothiolase with acetoacetyl CoA as the substrate has revealed as much as 12 percent of normal activity [6]. Most patients have no demonstrable enzyme activity and no immunoreactive cross-reacting material (CRM) protein [4].

There are three 3-oxothiolases in mammalian tissues, two of them mitochondrial and one cytoplasmic [7]. The cytoplasmic enzyme is involved in the synthesis of 3-oxoacyl compounds. The other mitochondrial enzyme (EC 2.3.1.16) has a very broad range of substrates against which it is active (for example, 3-oxohexanoylCoA), while the enzyme that is defective in this disorder has a high degree of substrate specificity, restricted to acetoacetylCoA and 2-methylacetoacetylCoA. A further distinctive property of this enzyme is its enhancement by potassium ions of its activity against acetoacetyl CoA [7]. It is the only enzyme with appreciable activity towards 2-methylacetoacetyl CoA. Thus, when homogenates derived from patients are tested using 2-methylacetoacetyl CoA as substrate, virtually no activity has been observed; in the presence of acetoacetyl CoA a depressed level of activity is found, reflecting the activities of the cytoplasmic and the nonspecific mitochondrial thiolase. Activation of cleavage of acetoacetyl CoA by potassium is not seen, providing further evidence of deficiency of the short chain-length-specific mitochondrial thiolase (EC 2.3.1.9). The K^+ -stimulated activity against the 2-methyl-acetoacetyl CoA has been 0–4 percent of control [8, 15, 19, 25, 26]. A patient with a less severe phenotype had 7 percent of control activity [19], but in general enzyme activity has not correlated with clinical severity. A coupled assay for fibroblast extracts in which $\text{NaH}^{14}\text{CO}_3$ is fixed ultimately to methylmalonyl CoA [41] revealed activity of 2 percent of controls in five patients and 20 percent of control in the milder patient [19].

The enzyme in liver is a 176-kDa tetramer of four identical subunits. A precursor enzyme is imported into the mitochondria where a leader peptide is removed to release the tetrapeptide. Immunochemical studies of the enzyme protein have revealed considerable heterogeneity, including no evidence of enzyme by immunoblot or by pulse chase and evidence of an unstable protein by the presence of detectable protein following a 1-hour pulse that was gone after a 6-hour chase [42–44].

Complementation analysis of seven patients with thiolase deficiency, in which the conversion of ^{14}C -labeled isoleucine to glutamate and aspartate of cellular protein was the measure of activity, revealed evidence of three complementation groups [45]. Assessment of the degradative pathways of isoleucine by study of the incorporation of ^{14}C -2-methylbutyrate into macromolecules in intact cells revealed low activity in nine patients with clinically severe phenotypes [46], and over 30 percent of control activity in patients with milder disease.

The gene (*ACAT1*) has a cDNA of 1518 bp in 12 exons and encodes a 427 amino acid precursor protein [4, 10, 47–50]. The sequence is of a 33 residue leader sequence and a 394 amino acid mature enzyme of 41,385 molecular weight. In a study of four patients, the length of the mRNA was normal. The amounts of mRNA were reduced in two and normal in two [49]. Many patients, to date, have been compounds of two mutations. For instance, in a German family, a G to A mutation at position 1138 led to a change from alanine to threonine at amino acid 347 of the mature enzyme [50]; this is a highly conserved region of the enzyme. This allele was inherited from one parent; the mutation in the other parent led to no mRNA expression. In another family a G to A mutation at 547 led to a glycine to arginine change at position 150; the other allele in this patient was altered at a splice site leading to skipping of exon 8 [10]. This individual was the asymptomatic father of a patient with severe attacks of ketoacidosis [5] who received the G to A 547 mutation from his father and an AG to CG transition at a splice site in exon 10 that led to skipping of exon 11 [11].

In a series of 26 patients, there were 45 independent chromosomes and 30 different mutations [4]. Only five were homozygous mutant. Expression analysis and the nature of mutation indicated that 24 of the mutations coded for absent activity. Only a minority of mutations led to appreciable residual activity. This would be expected to lead to a milder clinical phenotype, as was seen in one of the homozygotes who had the G145E mutation. The extensive heteroallelic nature of mutation was associated with a general absence of genotype–phenotype correlation. Mutant siblings had different clinical phenotypes. *In vitro* evidence of residual activity in fibroblasts was a better predictor of relatively mild disease. An absence of tiglylglycine in the urine suggests the presence of residual activity *in vitro* and was often associated with mild disease. Its absence does not exclude a severe phenotype, but its presence is a useful predictor of severe disease.

TREATMENT

The considerable heterogeneity in the severity of clinical presentation indicates that there should be individual programs of treatment. Nevertheless, it would appear prudent to restrict the intake of isoleucine in any patient with this disorder. Long fasting should be avoided [27], especially during intercurrent febrile illness or intestinal upset. In this situation, intravenous glucose is indicated and may abort a ketoacidotic crisis.

The acute acidotic attack should be treated with copious amounts of water and electrolytes (Chapter 2). Carnitine is useful intravenously in the acute situation and orally long term in order to esterify and remove tiglyl CoA and the other accumulated CoA esters.

DIFFERENTIAL DIAGNOSIS: RELATED DISORDERS OF KETOLYSIS

Patients have been observed in whom the clinical picture and pattern of excretion of 2-methyl-3-hydroxybutyric acid and tiglylglycine are typical of 3-oxothiolase deficiency, but the activity of the thiolase enzyme is normal (Figure 13.10).



Figure 13.10 ZM: A boy with repeated episodes of vomiting and ketoacidosis, along with tiglylglycinuria and 2-methyl-3-hydroxybutyric aciduria which increased on administration of isoleucine, consistent with 3-oxothiolase deficiency. However, assay of the enzyme revealed normal activity. The incorporation of 1-¹⁴C-2-methylbutyrate into macromolecules was 24 percent of control; in a series of patients with 3-oxothiolase deficiency the range was 2–31 percent of control.

The patient illustrated was studied by Iden and colleagues [46] in their assessment of the pathway from ¹⁴C-2-methylbutyrate to macromolecules, and an abnormality was demonstrated in the pathway that was equivalent to the milder patients with thiolase deficiency, indicating the presence of a defect in the pathway not yet identified.

Cytosolic acetoacetyl CoA thiolase deficiency has been reported in two patients with hypotonia and delayed development [51, 52]. One was also ataxic and choreic. One patient had elevated levels of acetoacetate in blood and urine [51]; the other did not, but had elevated levels of lactate and pyruvate and a low lactate to pyruvate ratio [50]. Abnormalities were found in the cytosolic thiolase, and the synthesis of cholesterol from acetate was reduced. Ketosis was reduced by restriction of fat intake, but there was no clinical improvement.

Succinyl CoA:3-oxoacid CoA transferase (SCOT) (EC2.8.3.5) deficiency has been reported in patients [53–56] who presented with ketoacidosis in infancy and had repeated episodes of ketoacidosis with infections. Two patients died, as had four siblings. Activity of the transferase enzyme in fibroblasts was deficient, virtually completely in the patients who died [54, 56] and 20 percent of control in the survivor. This enzyme catalyzes the conversion of acetoacetic acid to acetoacetyl CoA. It is thus a key enzyme in the utilization of acetoacetate and 3-hydroxybutyrate formed in liver and transported in the blood to peripheral tissues. The organic acid analysis of the urine revealed only 3-hydroxybutyrate, acetoacetate, and 3-hydroxyisovaleric, all consistent with ketosis. The diagnosis may be suspected by the persistence of ketosis in the steady-state fed condition, but it is now clear that not all patients have steady-state ketonuria. The gene for SCOT cDNA and gene have been cloned [57, 58]. At least ten mutations have been identified [59]. Missense mutations have been documented to be causative of deficient enzyme assay by expression analysis in SV40 transformed SCOT-null fibroblasts [58]. A novel mutation (R268H) was found to have considerable residual activity in expression analysis at 37°C, but was temperature sensitive, with only 4 percent of control activity at 40°C [59]. Thus activity in expression analysis may not reflect the condition in the patient.

REFERENCES

1. Daum RS, Lamm PH, Mamer OA, Scriver CR. A 'new' disorder of isoleucine catabolism. *Lancet* 1971; **2**: 1289.
2. Daum RS, Scriver CR, Mamer OA *et al*. An inherited disorder of isoleucine catabolism causing accumulation of α -methylacetoacetate and α -methyl- β -hydroxybutyrate and intermittent metabolic acidosis. *Pediatr Res* 1973; **7**: 149.
3. Pollitt RJ. The occurrence of substituted 3-methyl-3-hydroxyglutaric acids in urine in propionic acidemia and in β -ketothiolase deficiency. *Biomed Mass Spectrom* 1983; **4**: 253.
4. Fukao T, Scriver CR, Kondo N *et al*. The clinical phenotype and outcome of mitochondrial acetoacetyl-CoA thiolase deficiency

- (β -ketothiolase or T2 deficiency) in 26 enzymatically proved and mutation-defined patients. *Mol Genet Metab* 2001; **72**: 109.
5. Saudubray JM, Specola N. Ketolysis defects. In: Fernandes J, Saudubray JM, Tada K (eds). *Inborn Metabolic Diseases*. Berlin: Springer-Verlag, 1990: 411.
 6. Schutgens RBH, Middleton B, Blij JF *et al.* Beta-ketothiolase deficiency in a family confirmed by in vitro enzymatic assays in fibroblasts. *Eur J Pediatr* 1982; **139**: 39.
 7. Middleton B. The oxoacyl-coenzyme A thiolases of animal tissues. *Biochem J* 1973; **132**: 717.
 8. Middleton B, Bartlett K. The synthesis and characterization of 2-methylacetoacetyl coenzyme A and its use in the identification of the site of the defect in 2-methyl-acetoacetic and 2-methyl-3-hydroxybutyric aciduria. *Clin Chim Acta* 1983; **128**: 291.
 9. Fukao T, Yamaguchi S, Nagasawa H *et al.* Molecular cloning of cDNA for human mitochondrial acetoacetyl-CoA thiolase and molecular analysis of 3-ketothiolase deficiency. *J Inherit Metab Dis* 1990; **13**: 757.
 10. Masuno M, Kano M, Fukao T *et al.* Chromosome mapping of the human mitochondrial acetoacetyl-CoA thiolase gene to band 11q223-q231 by fluorescence *in situ* hybridization. *Cytogenet Cell Genet* 1992; **60**: 121.
 11. Fukao T, Yamaguchi S, Orii T *et al.* Identification of three mutant alleles of the gene for mitochondrial acetoacetyl-Coenzyme A thiolase. *J Clin Invest* 1992; **89**: 474.
 12. Fukao T, Yamaguchi S, Orii T *et al.* Molecular basis of 3-ketothiolase deficiency: Identification of an AG to AC substitution at the splice acceptor site of intron 10 causing exon 11 skipping. *Biochim Biophys Acta* 1992; **1139**: 184.
 13. Sakurai S, Fukao T, Haapalainen AM *et al.* Kinetic and expression analyses of seven novel mutations in mitochondrial acetoacetyl-CoA thiolase (T2): identification of a Km mutant and an analysis of the mutational sites in the structure. *Mol Genet Metab* 2007; **90**: 370.
 14. Robinson JP, Feigin RD, Tenenbaum SM, Hillman RE. Hyperglycinemia with ketosis due to a defect in isoleucine catabolism. *Pediatrics* 1972; **50**: 890.
 15. Hillman RE, Keating JP. Ketothiolase deficiency as a cause of the ketotic hyperglycinemia syndrome. *Pediatrics* 1974; **53**: 221.
 16. Gompertz D, Saudubray JM, Charpentier C *et al.* A defect in isoleucine metabolism associated with α -methyl- β -hydroxybutyric acid and α -methylacetoacetic aciduria. Quantitative *in vivo* and *in vitro* studies. *Clin Chim Acta* 1974; **57**: 269.
 17. Robinson BH, Sherwood G, Taylor J *et al.* Acetoacetyl CoA thiolase deficiency. A cause of severe ketoacidosis in infancy simulating salicylism. *J Pediatr* 1979; **95**: 228.
 18. Henry CG, Strauss AW, Keating JP, Hillman RE. Congestive cardiomyopathy associated with β -ketothiolase deficiency. *J Pediatr* 1981; **99**: 754.
 19. Middleton B, Bartlett K, Romanos A *et al.* 3-Ketothiolase deficiency. *Eur J Pediatr* 1986; **144**: 586.
 20. Hartlage P, Eller G, Carter L *et al.* Mitochondrial acetoacetyl-CoA thiolase deficiency. *Biochem Med Metab Biol* 1986; **36**: 198.
 21. Saudubray JM, Specola N, Middleton B *et al.* Hyperketotic states due to inherited defects of ketolysis. *Enzyme* 1987; **38**: 80.
 22. Halvorsen S, Stokke O, Jellum E. A variant form of 2-methyl-3-hydroxybutyric and 2-methylacetoacetic aciduria. *Acta Paediatr Scand* 1979; **68**: 123.
 23. Hiyama K, Sakura N, Matsumoto T, Kuhara T. Deficient beta-ketothiolase activity in leukocytes from a patient with 2-methylacetoacetic aciduria. *Clin Chim Acta* 1986; **155**: 189.
 24. Leonard JV, Middleton B, Seakins JWT. Acetoacetyl CoA thiolase deficiency presenting as ketotic hypoglycemia. *Pediatr Res* 1987; **21**: 211.
 25. Middleton B, Gray RGF, Bennett MJ. Two cases of beta-ketothiolase deficiency: a comparison. *J Inherit Metab Dis* 1984; **7**: 131.
 26. Ozand PT, Rashed MR, Gascon GG *et al.* 3-Ketothiolase deficiency: a review and four new patients with neurologic symptoms. *Brain Dev* 1994; **16**(Suppl.): 38.
 27. Sovik O. Mitochondrial 2-methylacetoacetyl-CoA thiolase deficiency: an inborn error of isoleucine and ketone body metabolism. *J Inherit Metab Dis* 1993; **16**: 46.
 28. Gibson KM, Feigenbaum ASJ. Phenotypically mild presentation in a patient with 2-methylacetoacetyl-coenzyme A (beta-keto) thiolase deficiency. *J Inherit Metab Dis* 1997; **20**: 712.
 29. Sebetta G, Bachmann C, Giardini O *et al.* Beta-ketothiolase deficiency with favourable evolution. *J Inherit Metab Dis* 1987; **10**: 405.
 30. Sewell AC, Herwig J, Wiegatz I *et al.* Mitochondrial acetoacetyl-CoA thiolase (beta-ketothiolase) deficiency and pregnancy. *J Inherit Metab Dis* 1998; **21**: 441.
 31. Mrazova L, Fukao T, Halovd K *et al.* Two novel mutations in mitochondrial acetoacetyl-CoA thiolase deficiency. *J Inherit Metab Dis* 2005; **28**: 235.
 32. Merinero B, Perez-Cerda C, Garcia MJ *et al.* Two siblings with different clinical conditions. *J Inherit Metab Dis* 1987; **10**(Suppl. 2): 276.
 33. Landaas S. Accumulation of 3-hydroxyisobutyric acid 2-methyl-3-hydroxybutyric acid and 3-hydroxyisovaleric acid in ketoacidosis. *Clin Chim Acta* 1975; **64**: 143.
 34. Sweetman L, Weyler W, Nyhan WL *et al.* Abnormal metabolites of isoleucine in a patient with propionyl-CoA carboxylase deficiency. *Biomed Mass Spectrom* 1978; **5**: 198.
 35. Aramaki S, Lehotay D, Sweetman L *et al.* Urinary excretion of 2-methylacetoacetate 2-methyl-3-hydroxybutyrate and tiglylglycine after isoleucine loading in the diagnosis of 2-methylacetoacetyl-CoA thiolase deficiency. *J Inherit Metab Dis* 1991; **14**: 63.
 36. Duran M, Bruinvis L, Ketting D *et al.* The identification of (E)-2-methylglutaconic acid a new isoleucine metabolite in the urine of patients with β -ketothiolase deficiency propionic acidemia and methylmalonic academia. *Biomed Mass Spectrom* 1982; **9**: 1.
 37. Rashed MR, Ozand PT, Bucknall M, Little D. Diagnosis of inborn errors of metabolism from blood spots by acylcarnitines and amino acids profiling using automated electrospray tandem mass spectrometry. *Pediatr Res* 1995; **38**: 324.

38. Monastiri K, Amri F, Limam K *et al.* Beta-ketothiolase (2-methylacetoacetyl-CoA thiolase) deficiency: a frequent disease in Tunisia? *J Inherit Metab Dis* 1999; **22**: 932.
39. Jakobs C, Sweetman L, Nyhan WL. Hydroxy acid metabolites of branched-chain amino acids in amniotic fluid. *Clin Chim Acta* 1984; **140**: 157.
40. Middleton B. Identification of heterozygotes for the defect of mitochondrial 3-ketothiolase causing 2-methyl-3-hydroxy-butyric aciduria. *J Inherit Metab Dis* 1987; **10**(Suppl. 2): 270.
41. Gibson KM, Lee CF, Kamali V, Sovik O. A coupled assay detecting defects in fibroblast isoleucine degradation distal to enoyl-CoA hydratase: application to 3-oxothiolase deficiency. *Clin Chim Acta* 1992; **205**: 127.
42. Nagasawa H, Yamaguchi S, Orii T *et al.* Heterogeneity of defects in mitochondrial acetoacetyl-CoA thiolase biosynthesis in fibroblasts from four patients with 3-ketothiolase deficiency. *Pediatr Res* 1989; **26**: 145.
43. Yamaguchi S, Orii T, Sakura N *et al.* Defect in biosynthesis of mitochondrial acetoacetyl-coenzyme A thiolase in cultured fibroblasts from a boy with 3-ketothiolase deficiency. *J Clin Invest* 1988; **81**: 813.
44. Hiyama K, Sakura N, Matsumoto T *et al.* Deficient beta-ketothiolase activity in leukocytes from a patient with 2-methylacetoacetic aciduria. *Clin Chem Acta* 1986; **155**: 189.
45. Sovik O, Saudubray JM, Munnich A, Sweetman L. Genetic complementation of analysis of mitochondrial 2-methylacetoacetyl-CoA thiolase deficiency in cultured fibroblasts. *J Inherit Metab Dis* 1992; **15**: 359.
46. Iden P, Middleton B, Robinson B *et al.* 3-Oxothiolase activities and [^{14}C]-2-methylbutanoic acid incorporation in cultured fibroblasts from 13 cases of suspected 3-oxothiolase deficiency. *Pediatr Res* 1990; **28**: 518.
47. Yamaguchi S, Fukao T, Nagasawa H *et al.* 3-Ketothiolase deficiency: molecular heterogeneity of the enzyme defect and cloning of the cDNA. *Prog Clin Biol Res* 1990; **321**: 673.
48. Fukao T, Kamijo K, Osumi T *et al.* Molecular cloning and nucleotide sequencing of cDNA encoding the entire precursor of rat mitochondrial acetoacetyl-CoA thiolase. *J Biochem* 1989; **106**: 197.
49. Fukao T, Yamaguchi S, Kano M *et al.* Molecular cloning and sequence of the complementary DNA encoding human mitochondrial acetoacetyl-coenzyme A thiolase and study of the variant enzymes in cultured fibroblasts from patients with 3-ketothiolase deficiency. *J Clin Invest* 1990; **86**: 2086.
50. Fukao T, Yamaguchi S, Tomatsu S *et al.* Evidence for a structural mutation (347Ala to Thr) in a German family with 3-ketothiolase deficiency. *Biochem Biophys Res Commun* 1991; **179**: 124.
51. De Groot CJ, Luit-De Haan G, Hulstaert CE, Hommes FA. A patient with severe neurologic symptoms and acetoacetyl-CoA thiolase deficiency. *Pediatr Res* 1977; **11**: 1112.
52. Bennett MJ, Hosking GP, Smith MF *et al.* Biochemical investigations on a patient with a defect in cytosolic acetoacetyl-CoA thiolase associated with mental retardation. *J Inherit Metab Dis* 1984; **7**: 125.
53. Cornblath M, Gingell RL, Fleming GA *et al.* A new syndrome of ketoacidosis in infancy. *J Pediatr* 1971; **79**: 413.
54. Spence MW, Murphy MG, Cook HW *et al.* Succinyl-CoA: 3-ketoacid CoA-transferase deficiency. A 'new' phenotype? *Pediatr Res* 1973; **7**: 394.
55. Middleton B, Day R, Lombers A, Saudubray JM. Infantile ketoacidosis associated with decreased activity of succinyl-CoA: 3-ketoacid CoA-transferase. *J Inherit Metab Dis* 1987; **10**(Suppl. 2): 273.
56. Tildon JT, Cornblath M. Succinyl-CoA: 3-ketoacid CoA transferase deficiency. *J Clin Invest* 1972; **51**: 493.
57. Chen MF, Robert C, Perez-Cerda M *et al.* Succinyl CoA:3-oxoacid CoA transferase (SCOT): human cDNA cloning, human chromosomal mapping to 5p13, and mutation detection in a SCOT-deficient patient. *Am J Hum Genet* 1996; **59**: 519.
58. Fukao T, Mitchell GA, Song XQ *et al.* Succinyl-CoA:3-ketoacid CoA transferase (SCOT: cloning of the human SCOT gene, tertiary structural modelling of the human SCOT monomer, and characterization of three pathogenic mutations). *Genome* 2000; **68**: 144.
59. Fukao T, Kursula P, Owen EP *et al.* Identification and characterization of a temperature-sensitive R268H mutation in the human succinyl-CoA:3-ketoacid CoA transferase (SCOT) gene. *Mol Genet Metab* 2007; **92**: 216.

DISORDERS OF AMINO ACID METABOLISM

14.	Alkaptonuria	105
15.	Phenylketonuria	112
16.	Hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin	123
17.	Biogenic amines	136
18.	Homocystinuria	144
19.	Maple syrup urine disease (branched-chain oxoaciduria)	152
20.	Oculocutaneous tyrosinemia/tyrosine aminotransferase deficiency	164
21.	Hepatorenal tyrosinemia/fumarylacetoacetate hydrolase deficiency	171
22.	Nonketotic hyperglycinemia	180

Alkaptonuria

Introduction	105	Treatment	109
Clinical abnormalities	105	References	110
Genetics and pathogenesis	108		

MAJOR PHENOTYPIC EXPRESSION

Deposits of dark pigment in the sclerae, cartilage and skin, early osteoarthritis; dark urine, homogentisic aciduria, and defective activity of homogentisic acid oxidase.

INTRODUCTION

Alkaptonuria was recognized by Garrod [1, 2] as an inborn error of metabolism, around the beginning of the twentieth century. In fact, it was out of his studies of patients with alkaptonuria and their families that he conceived the idea that inborn errors of metabolism result from alterations, each in a single enzyme that is itself the consequence of a single genetic event. This was the first enunciation of what came to be known as the one-gene-one-enzyme hypothesis [3].

Alkaptonuria, or the excretion of urine which darkens on exposure to oxygen, is the result of the excretion of large amounts of homogentisic acid. The material precipitated by Boedeker [4] as the lead salt was identified by Wolkow and Baumann [5] as 2,5-dihydroxyphenylacetic acid and named homogentisic acid, as a similar structure to gentisic acid, 2,5-dihydroxyphenylbenzoic acid.

Alkaptonuria is usually asymptomatic for many years. Its major clinical effects are on the cartilaginous surfaces of the joints. Resulting osteoarthritis may be debilitating. The disease has been present since antiquity; evidence of the disease has been reported in an Egyptian mummy who lived around 1500BC [6]. Patients may also develop calcification of the aortic valves. Calculi of the urinary tract and of the prostate are relatively common.

Homogentisic acid is a normal intermediate in the catabolism of the aromatic amino acids, phenylalanine and tyrosine (Figure 14.1). It accumulates because of a defective activity of homogentisic acid oxidase [7]. This

enzyme, which in mammalian systems is found only in liver and kidney, has been shown to be defective in both tissues in alkaptonuria. It catalyzes the conversion of homogentisic acid to maleylacetoacetic acid, which is ultimately converted to fumaric and acetoacetic acids. The gene for homogentisic acid oxidase has been cloned and mapped to chromosome 3q21-23 [8, 9]. The gene contains 14 exons over 60 Kb of genomic DNA [10]. A number and variety of mutations have been identified [10–12].

A promising therapy involves treatment with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione, or NTBC (nitisinone) [13, 14]. This herbicide inhibits 4-hydroxyphenylpyruvate dioxygenase, the enzyme that produces homogentisic acid. NTBC is approved for the treatment of hepatorenal tyrosinemia (Chapter 26). It should be even more effective in alkaptonuria.

CLINICAL ABNORMALITIES

The urine of an alkaptonuric individual usually appears normal when passed. It turns dark on standing, but most people do not leave their urine standing around to be observed, so most individuals live many years, usually well into adulthood, without recognizing that they are alkaptonuric. The addition of alkali to the urine will cause the pigment to appear more rapidly (Figure 14.2) [4]. Infants have been recognized because their cloth diapers, which had been washed with an alkaline soap or detergent, turned black or brown when they became wet with urine.

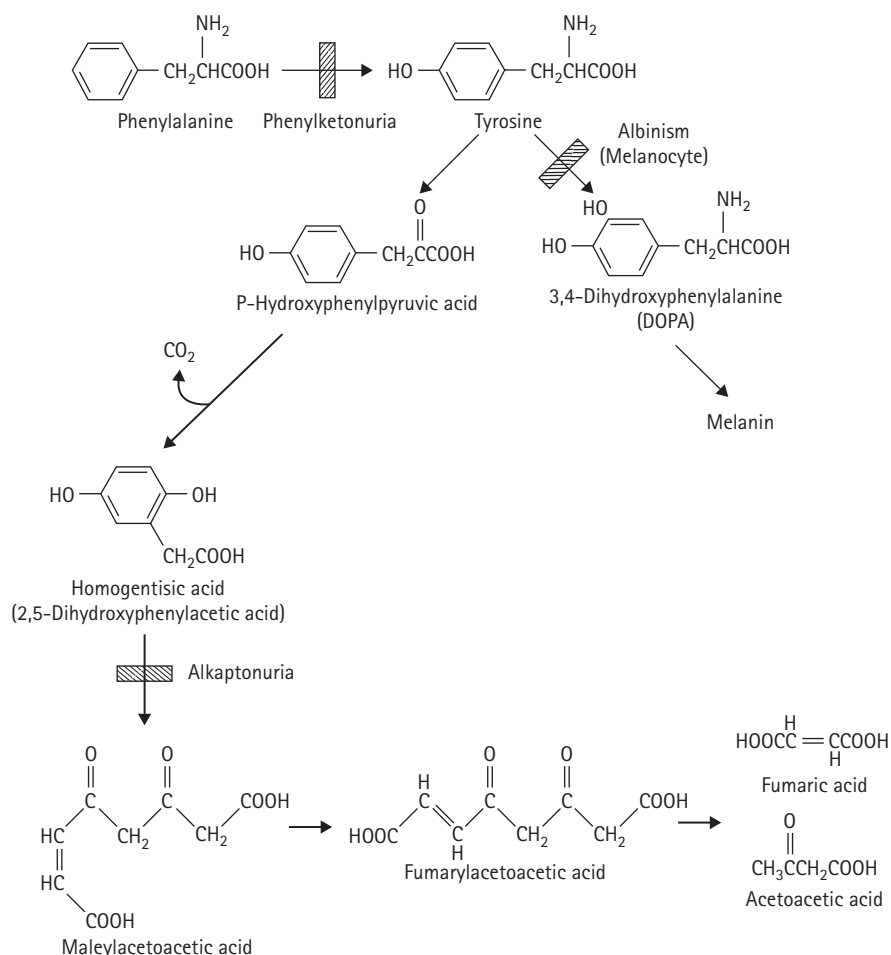


Figure 14.1 Aromatic amino acid metabolism. The site of the defect in alkaptonuria is in the homogentisic acid oxidase.



Figure 14.2 Alkaptonuric urine. The flask on the right contains fresh urine darkened somewhat; the flask on the left to which sodium hydroxide was added contains a black suspension.



Figure 14.3 Diaper of an infant with alkaptonuria. Cloth diapers washed in detergent turn black on contact with alkaptonuric urine. We find that disposable diapers like this one become pink or red.

Cloth diapers are seldom used today and instead we have observed a reddish discoloration from alkaptonuric urine in disposable diapers (Figure 14.3).

In some patients, the diagnosis is suggested by a positive test for urinary-reducing substance, a feature that was also recognized in 1859 [4]. The urine does not contain glucose, and so laboratories that test urine only with glucose

oxidase will miss this opportunity to find alkaptonuria. Homogentisic acid reduces the silver in a photographic emulsion, and alkaptonuric urine may be used to develop a photograph, providing a dramatic qualitative and even quantitative test for the disease [15, 16]. Homogentisic acid may be identified by paper chromatography, and there is a specific enzymatic analysis that permits quantification

[17]. We more often find it first on analysis of the urine for organic acids [18]. We also developed a high performance liquid chromatography (HPLC) method for the quantitation of homogentisic acid and its products [19]. An adult with alkaptonuria excretes as much as 4–8 g of homogentisic acid daily [20]. The compound is excreted so efficiently that little is found in the plasma, although the amounts found by stable isotope internal standard gas chromatography mass spectrometry are considerably higher than those of normal plasma [21].

Patients with alkaptonuria have no symptoms as children or young adults. With age, they develop pigmentation of the sclerae or cartilage of the ear (Figures 14.4 and 14.5). The condition of widespread deposition of pigment in alkaptonuria was first called 'ochronosis' by Virchow [22] because the gray, blue, or black pigment appeared ochre under the microscope. These pigment deposits should be visible by 30 years of age. Actually, deposition may be widespread throughout the cartilage and fibrous tissue of

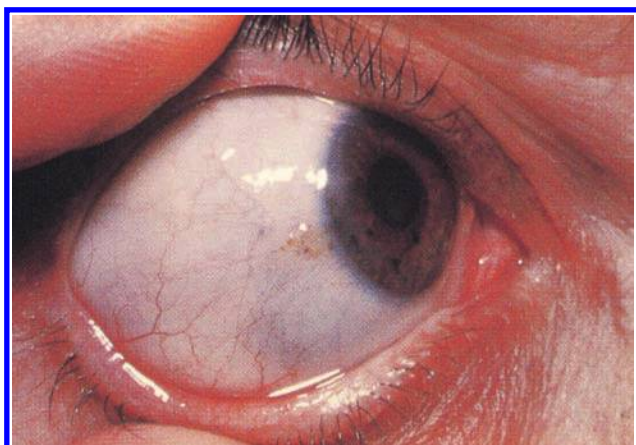


Figure 14.4 Ochronotic pigment in the sclera.

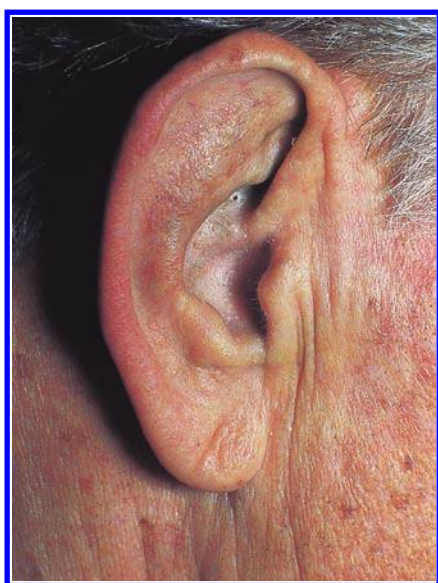


Figure 14.5 Ochronotic pigment in the cartilage of the ear.



Figure 14.6 Ochronotic pigment has been deposited diffusely over the nose of this 52-year-old man.



Figure 14.7 The same patient had fine, stippled pigment over the dorsum of his hands.

the body [23–27]. Pigment may be seen at surgery and of course the diagnosis may become apparent first in this way with the rapid formation of pigment on exposure of tissues to air [28]. Pigment may be seen in the buccal mucosa and the nails. There may be deposits in the skin (Figures 14.6 and 14.7), leading to areas of dusky coloration of the skin. In addition to those shown, the cheeks, forehead, axillae, and genital regions may be involved. The sweat may be dark and the cerumen brown or black.

The benign early course of these patients contrasts sharply with the severity of the ochronotic arthritis that develops early in adult life (Figure 14.8) [14, 22–24, 29–31]. The roentgenographic picture is of severe osteoarthritis (Figures 14.9 and 14.10), developing much earlier than in nonalkaptonuric individuals. Some clinical features are reminiscent of rheumatoid arthritis, because there are acute periods of inflammation. Early symptoms may be in the hip or knee – large weight-bearing joints, but back pain is often the earliest complaint [14]. Limitation of motion is seen early. Ultimately, marked limitation of motion is the rule and ankyloses are common. The arthritis has been



Figure 14.8 Knee of patient AB with ochronotic arthritis



Figure 14.9 Roentgenogram of the hip illustrating the advanced, early onset osteoarthritis characteristic of this disease.

noted to be earlier in onset and greater in severity in males, although the incidence in the two sexes is equal [32].

The roentgenographic appearance may be pathognomonic (Figures 14.9 and 14.10) [23, 33]. The intervertebral disks undergo marked degeneration. There may be rupture of an intervertebral disk. The disk spaces become narrow and calcium is deposited. There is a variable degree of fusion of the vertebral bodies. The bamboo-like appearance (Figure 14.10) is diagnostic of ochronotic arthritis. In contrast to rheumatoid disease, there is little osteophyte formation or calcification of the intervertebral ligaments. Mean decrement in height approximates 8 cm [14]. In contrast to conventional osteoarthritis, the large joints at the hip and shoulder are commonly involved in ochronosis, whereas the sacroiliac joint may be uninvolved.



Figure 14.10 Roentgenogram of the osteoarthritic spine in the patient with alkaptonuria resembles bamboo.

In the involved joints, there are degenerative osteoarthritic changes, occasional free intra-articular bodies [34], and calcification of the surrounding tendons. The arthritis of this disease is disabling. The patient may become bedridden or chairbound. Calcification of the ear cartilage is another roentgenographic characteristic of the disease.

Torn muscles or tendons indicate connective tissue disease [14], and thickening of the Achilles tendon is characteristic. Many have effusions of joints or the suprapatellar bursa.

Urinary tract calculi are common late findings, appearing at a mean of 64 years [14]. Urinary tract stones may also be seen in patients less than 15 years of age and even as young as two years [35]. Calculi in the prostate are very common in men over 60 years [14].

Patients with alkaptonuria commonly have heart disease. Mitral and aortic valvulitis may be seen at autopsy. Aortic dilation or calcification of the aortic and mitral valve is common [14]. Myocardial infarction is a common cause of death and there may be coronary artery calcification [14].

Index of the inflammatory nature of the disease is elevated sedimentation rate ranging from 55 to 110 mm/hour [14]. Levels of osteocalcin are elevated in some patients, representing the formation of new bone [14], and urinary collagen N-telopeptide. An index of bone resorption is also elevated.

GENETICS AND PATHOGENESIS

Alkaptonuria is inherited as an autosomal recessive trait [36]. Consanguinity was originally noted by Garrod [1] and subsequently by others [37]. Heterozygote detection should be possible by assay of the enzyme in biopsied

liver, but this has not been done. Cloning of the gene for homogentisic acid oxidase in man [7, 8] has permitted molecular studies in patients with alkaptonuria and their families.

A majority of patients has represented compounds of two different mutations. In one series [14], mutations were defined in 90 percent of alleles tested. Most have been missense, but nonsense mutations and intronic mutations resulting in frame shift have been observed. Mutations have been identified in every exon, except 14. There has been some clustering in exons 7 to 10 [12]. One mutation (M368V) has been observed in at least one allele in 14 patients [14]. Correlation between genotype and phenotype has not been apparent. In a highly consanguineous Turkish population, a frequent mutation was R225H [38] which has also been found in Spanish patients. A considerable heterogeneity of mutation has been observed in the populations studied [39, 40]. They are not randomly distributed, but occur in more than a third of patients in CCC repeats and in the inverted complement GGG. Hot spots for mutation in this gene are in these triplets, not in CpG dinucleotides.

The disease is common in Slovakia [41]. Incidence has been estimated at one in 19,000. Ten different mutations have been found in this population, but two were found in more than half the chromosomes [41, 42]. These were c183-1G>A and glycine161arginine. Mutational information has been correlated with the complex crystalline structure of the oxidase enzyme consistent with interference with folding by single nucleotide substitution [43].

Abnormality in the gene determines very low activity of homogentisic acid oxidase [17]. Defective activity of this enzyme has been documented in autopsied kidney [44]. In normal individuals, intravenously administered ^{14}C -labeled homogentisic acid is oxidized rapidly to $^{14}\text{CO}_2$, while in alkaptonuric individuals 90 percent is excreted unchanged in the urine [45].

The arthritis and other ochronotic elements of the disease are thought to result from the binding of highly

cartilage [46]. Benzoquinone-acetic acid and p-quinones in general form 1,4 addition products with sulfhydryl and amino groups [47].

TREATMENT

For many years, no treatment has been successful in reducing the accumulation of homogentisic acid or interfering with its late effects on tissues. We have shown that it is possible to reduce the formation of homogentisic acid by reducing the intake of phenylalanine and tyrosine, and this is relatively easy in an infant [15]. However, compliance with a rather difficult diet would be a major problem in this disorder in which the symptoms are so many years in the future. Dietary reduction also reduced the excretion of homogentisic acid in a 45-year-old patient, but the authors judged it impractical [48]. Another approach was to employ reducing agents, such as ascorbic acid, in an attempt to prevent the oxidation of homogentisic acid to benzoquinone-acetic acid. Homogentisic acid inhibits the growth of cultured human articular chondrocytes. Ascorbic acid prevents this effect, and also prevents the binding of ^{14}C -homogentisic acid to connective tissues in rats [49]. We demonstrated that treatment of alkaptonuric patients with ascorbic acid was associated with a complete disappearance of benzoquinone-acetic acid from the urine. Similar results were reported by Mayatepek *et al.* [48].

Therapy with NTBC represents an advance toward rational therapy because the compound inhibits the enzyme directly before homogentisic acid in the pathway of tyrosine catabolism (Figure 14.1). Extensive experience in hepatorenal tyrosinemia (Chapter 22) indicated its surprising safety. Toxicity would be the reproduction of symptoms of oculocutaneous tyrosinemia (Chapter 21) and photophobia and corneal crystals have been observed in infants with hepatorenal tyrosinemia treated with NTBC [13]. In an experience with two adults [14] in whom diet was not modified, 0.7 and 1.4 mg every day reduced urinary homogentisic acid excretion to 0.13 and 1.4 g per day. Plasma levels of tyrosine rose to 715 and 1288 mmol/L. There were no ocular symptoms and slit lamp examination was normal.

In the most recent summary of this experience, 20 patients were treated with 2 mg/day of nitisinone [50] for up to three years. One patient developed subepithelial corneal lesions. There was a 95 percent reduction in urinary homogentisic acid. Nevertheless, there were no differences between treated patients and controls in such objective measurements as range of motion at the hip. This may mean that treatment should start earlier in life.

Arthritis, once developed, may require orthopedic treatment. In one series [14], 50 percent of patients underwent surgical replacement of at least a hip, knee, or shoulder. Mean age for joint replacement was 55 years [14]. Calcification of the aortic valve has required valve replacement in at least four patients [14, 51].

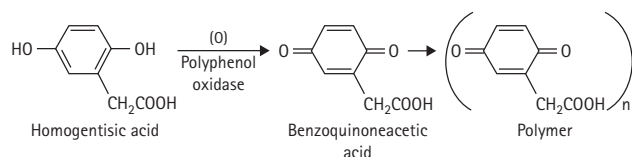


Figure 14.11 The oxidation of homogentisic acid to benzoquinoneacetic acid which precedes formation of the black polymer. Benzoquinoneacetic acid is a highly reactive compound. This, or a polymeric form, may bind to macromolecules in connective tissue.

reactive oxidation products of homogentisic acid to cartilage and other tissues. Homogentisic acid is oxidized to benzoquinone-acetic acid and ultimately to the polymeric ochronotic pigment (Figure 14.11). The reaction is catalyzed by an oxidase present in mammalian skin and

REFERENCES

- Garrod AE. The incidence of alkaptonuria: a study in chemical individuality. *Lancet* 1902; **2**: 1616.
- Garrod AE. *Inborn Errors of Metabolism*. London: Oxford University Press, 1908: 1523.
- Beadle GW, Tatum EL. Genetic control of biochemical reactions in neurospora. *Proc Natl Acad Sci USA* 1941; **27**: 499.
- Boedeker C. Ueber das Alcapton: ein Beitrag zur Frage: Welche Stoffe des Harns Konnen Kupferreduction bewirken. *Z Rat Med* 1859; **7**: 130.
- Wolkow M, Baumann E. Uber das Wesen der Alkaptonurie. *Z Physiol Chem* 1891; **15**: 228.
- Stenn FF, Milgratti JW, Lee S *et al*. Identification of homogenetic acid pigment in an Egyptian mummy. *Science* 1977; **197**: 566.
- La Du BN, Zannoni VG, Laster L, Seegmiller JE. The nature of the defect in tyrosine metabolism in alcaptonuria. *J Biol Chem* 1958; **230**: 251.
- Pollak MR, Chou Y-HW, Cerda JJ *et al*. Homozygosity mapping of the gene for alkaptonuria to chromosome 3 q2. *Nat Genet* 1993; **5**: 201.
- Janocha S, Wolz W, Srsen S *et al*. The human gene for alkaptonuria (AKU) maps to chromosome 3q. *Genomics* 1994; **15**: 5.
- Fernandez-Canon JM, Granadino B, Beltran-Valero de Bernabe D *et al*. The molecular basis of alkaptonuria. *Nat Genet* 1996; **14**: 15.
- Granadino B, Beltran-Valero de Bernabe D, Fernandez-Canon JM *et al*. The human homogentisate 12-dioxygenase (HGO) gene. *Genomics* 1997; **43**: 115.
- Beltran-Valero de Bernabe D, Granadino B, Chiarelli I *et al*. Mutation and polymorphism analysis of the human homogentisate 12-dioxygenase gene in alkaptonuria patients. *Am J Hum Genet* 1998; **62**: 776.
- Anikster Y, Nyhan WL, Gahl WA. NTBC in alkaptonuria. *Am J Hum Genet* 1998; **63**: 920.
- Phornphutkul C, Introne WJ, Perry MB *et al*. Natural history of alkaptonuria. *N Engl J Med* 2002; **347**: 2111.
- Fishberg EH. The instantaneous diagnosis of alkaptonuria on a single drop of urine. *J Am Med Assoc* 1942; **115**: 882.
- Neuberger A. Studies on alcaptonuria. I. The estimation of homogentisic acid. *Biochem J* 1947; **41**: 431.
- Seegmiller JE, Zannoni VG, Laster L, La Du BN. An enzymatic spectrophotometric method for the determination of homogentisic in plasma and urine. *J Biol Chem* 1961; **236**: 774.
- Hoffmann G, Aramaki S, Blum-Hoffmann E *et al*. Quantitative analysis for organic acids in biological samples: batch isolation followed by gas chromatographic/mass spectrometric analysis. *Clin Chem* 1989; **38**: 587.
- Wolff JA, Barshop B, Nyhan WL *et al*. Effects of ascorbic acid in alkaptonuria: alterations in benzoquinone acetic acid and an ontogenic effect in infancy. *Pediatr Res* 1989; **26**: 140.
- Neuberger A, Rimington C, Wilson JMG. Studies on alcaptonuria II. Investigations on a case of human alcaptonuria. *Biochem J* 1947; **41**: 438.
- Deutsch JC, Santhosh-Kumar CR. Quantitation of homogentisic acid in normal human plasma. *J Chromatogr B Biomed Appl* 1996; **677**: 147.
- Virchow R. Ein Fall von allgemeiner Ochronose der Knorpel und knorpelähnlichen Theile. *Arch Pathol Anat* 1866; **37**: 212.
- Bunim JJ, McGuire JS Jr, Hilbish TF *et al*. Alcaptonuria clinical staff conference at the National Institutes of Health. *Ann Intern Med* 1957; **47**: 1210.
- O'Brien WM, La Du BN, Bunim JJ. Biochemical pathological and clinical aspects of alcaptonuria ochronosis and ochronotic arthropathy. *Am J Med* 1963; **34**: 813.
- Cooper PA. Alkaptonuria with ochronosis. *Proc R Soc Med* 1951; **44**: 917.
- Minno AM, Rogers JA. Ochronosis: report of a case. *Ann Intern Med* 1957; **46**: 179.
- Osler W. Ochronosis: the pigmentation of cartilages sclerotics and skin in alkaptonuria. *Lancet* 1904; **1**: 10.
- Rose GK. Ochronosis. *Br J Surg* 1957; **44**: 481.
- O'Brien WM, La Du BN, Bunim JJ. Biochemical pathologic and clinical aspects of alcaptonuria: ochronosis and ochronotic arthropathy. *Am J Med* 1963; **34**: 813.
- Yules JH. Ochronotic arthritis: report of a case. *Bull N Engl Med Center* 1957; **16**: 168.
- O'Brien WM, Banfield WG, Sokoloff L. Studies on the pathogenesis of ochronotic arthropathy. *Arthritis Rheum* 1961; **4**: 137.
- Harrold AJ. Alkaptonuric arthritis. *J Bone Joint Surg (Br)* 1956; **38**: 532.
- Pomeranz MM, Friedman LJ, Tunick IS. Roentgen findings in alkaptonuric ochronosis. *Radiology* 1941; **37**: 295.
- Sutro CJ, Anderson ME. Alkaptonuric arthritis: cause for free intraarticular bodies. *Surgery* 1947; **22**: 120.
- Zibolen M, Srsheva K, Srsen S *et al*. Increased urolithiasis in patients with alkaptonuria in childhood. *Clin Genet* 2000; **58**: 79.
- Hogben L, Worrall RL, Zieve I. The genetic basis of alkaptonuria. *Proc R Soc Edinb (Biol)* 1932; **52**: 264.
- Khachadurian A, Abu Feisal K. Alkaptonuria: report of a family with seven cases appearing in four successive generations with metabolic studies in one patient. *J Chron Dis* 1958; **7**: 455.
- Uyguner O, Goicoechea de Jorge E, Cefle A *et al*. Molecular analysis of the HGO gene mutations in Turkish alkaptonuria patients suggest that the R58 fs mutation originated from Central Asia and was spread throughout Europe and Anatolia by human migrations. *J Inher Metab Dis* 2003; **26**: 17.
- Beltran-Valero de Bernabe D, Bernabe D, Jimenez FJ *et al*. Analysis of alkaptonuria (AKU) mutations and polymorphisms reveals that the CCC sequence motif is a mutational hot spot in the homogentisate 1,2 dioxygenase gene (HGO). *Am J Hum Genet* 1999; **64**: 1316.
- Beltran-Valero de Bernabe D, Peterson P, Luopajarvi K *et al*. Mutational analysis of the HGO gene in Finnish alkaptonuria patients. *J Med Genet* 1999; **36**: 922.
- Zatkova A, Chmelikova A, Polakova H *et al*. Rapid detection methods for five HGO gene mutations causing alkaptonuria. *Clin Genet* 2003; **63**: 145.

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42. Mueller CR, Fregin A, Srsen S *et al.* Allelic heterogeneity of alkaptonuria in Central Europe. *Eur J Hum Genet* 1999; **7**: 645.
 43. Rodriguez JM, Timm DE, Titus GP *et al.* Structural and functional analysis of mutations in alkaptonuria. *Hum Molec Genet* 2000; **9**: 2341.
 44. Zannoni VG, Seegmiller JE, La Du BN. Nature of the defect in alkaptonuria. *Nature* 1962; **153**: 952.
 45. Lustberg TJ, Schulman JD, Seegmiller JE. Metabolic fate of homogentisic acid-1-¹⁴C (HGA) in alkaptonuria and effectiveness of ascorbic acid in preventing experimental ochronosis. *Arthritis Rheum* 1969; **12**: 678.
 46. Zannoni VG, Lomtevas N, Goldfinger S. Oxidation of homogentisic acid to ochronotic pigment in connective tissue. *Biochim Biophys Acta* 1969; **177**: 94.
 47. Stoner R, Blivaiss BB. Homogentisic acid metabolism: a 14 addition reaction of benzoquinone-1-acetic acid with amino acids and other biological amines. *Fed Proc* 1965; **24**: 656.
 48. Mayatepek E, Kallas K, Anninos A, Muller E. Effects of ascorbic acid and low-protein diet in alkaptonuria. *Eur J Pediatr* 1998; **157**: 867.
 49. Lustberg TJ, Schulman JD, Seegmiller JE. Decreased binding of ¹⁴C-homogentisic acid induced by ascorbic acid in connective tissue of rats with experimental alcaptonuria. *Nature* 1970; **228**: 770.
 50. Introne WJ, Perry MB, Kayser MA *et al.* Nitisinone use in alkaptonuria: results of a three year trial. *Mol Genet Metab* 2010; **99**: 187 (Abstr.).
 51. Dereymaeker L, Van Parijs G, Bayart M *et al.* Ochronosis and alkaptonuria: report of a new case with calcified aortic valve stenosis. *Acta Cardiol* 1990; **45**: 87.

Phenylketonuria

Introduction	112	Treatment	118
Clinical abnormalities	113	Maternal PKU	120
Genetics and pathogenesis	115	References	120
Diagnosis	116		

MAJOR PHENOTYPIC EXPRESSION

Mental impairment, blue eyes, blond hair, fair skin, eczematous rash, vomiting in infancy, seizures, hyperactivity, unusual odor, positive urinary ferric chloride test, hyperphenylalaninemia, and deficiency of phenylalanine hydroxylase.

INTRODUCTION

Phenylketonuria (PKU) is a disorder of aromatic amino acid metabolism in which phenylalanine cannot be converted to tyrosine (Figure 15.1). The defective enzyme, phenylalanine hydroxylase, is expressed only in liver. This disease is a model for a public health approach to the control

of inherited disease since dietary treatment is effective in preventing impaired mental development. Routine neonatal screening programs have been most effective in the developed countries of the world. For these reasons, the full-blown picture of the classic disease is rarely observed today in these countries. Nevertheless, it does occur, and it is important that it be recognized.

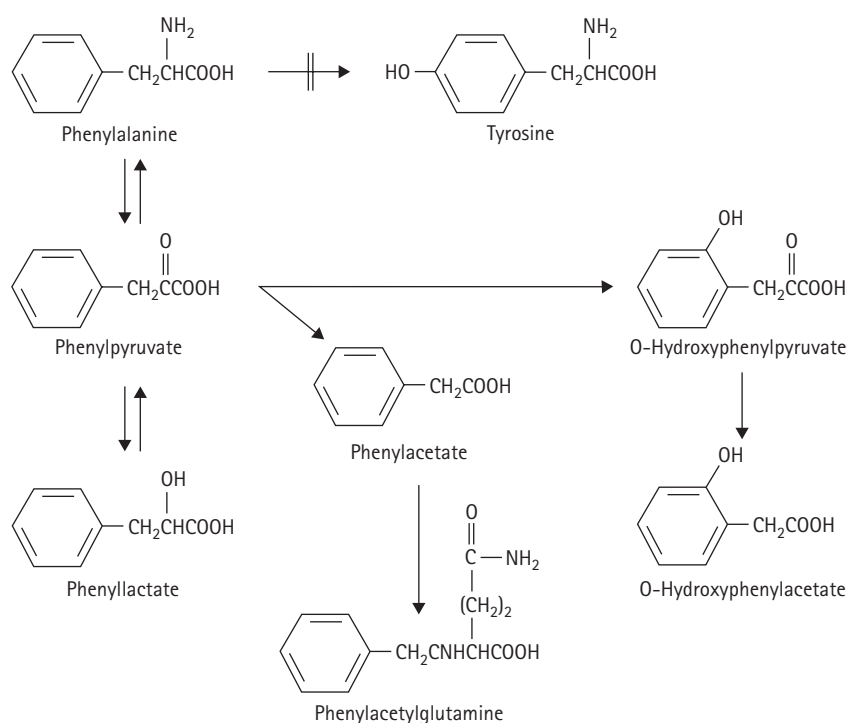


Figure 15.1 Metabolism of phenylalanine. The site of the defect in phenylketonuria is in phenylalanine hydroxylase. The compounds that accumulate as a consequence of the block are shown below.



Figure 15.2 A positive ferric chloride test in a patient with untreated phenylketonuria.

The disorder was discovered by Folling [1] who tested the urine of two siblings, brought to him by their mother, by the addition of ferric chloride and noted the deep green color that results from the presence of phenylpyruvic acid (Figures 15.1 and 15.2). The term 'phenylketonuria' was first proposed by Penrose [2] who recognized the disease as the first in which there was a chemical cause of mental impairment. The site of the molecular defect in the phenylalanine hydroxylase reaction was discovered by Jervis [3] who found that the conversion of phenylalanine to tyrosine could not be carried out *in vitro* by the preparations of liver obtained from patients with PKU. The gene coding for phenylalanine hydroxylase (PAH) has been identified and found to have 13 exons on chromosome 12, and a large number of mutations have been identified [4, 5]. A few mutant alleles account for a majority of human mutant chromosomes. Eight mutations have resulted in more than two-thirds of European mutant alleles.

CLINICAL ABNORMALITIES

The most important and sometimes the only manifestation of PKU is mental impairment (Figures 15.3, 15.4, 15.5, 15.6, 15.7, and 15.8). The intelligence of all but 1 percent of untreated patients is very low, with intelligence quotients (IQ) usually under 50 [6, 7]. A few patients with untreated PKU have had borderline intelligence.

Phenylketonuric infants appear normal at birth. Impaired mental development may not be evident for months. Vomiting may be a prominent early symptom. It may be severe enough to suggest a diagnosis of pyloric stenosis, and pyloromyotomy has been performed on such



Figure 15.3 The face of this patient with phenylketonuria illustrates the rather subtle eczematoid rash. The brown eyes remind us that all patients with this disease do not have blue eyes. In addition, he had epicanthal folds and a left internal strabismus.

patients [8, 9]. Irritability, an eczematoid rash (Figure 15.3), and an unusual odor may also be observed very early in life. The odor of the phenylketonuric patient is that of phenylacetic acid (Figure 15.1). It has variously been described as mousy, barny, wolf-like, or musty. Currently,



Figure 15.4 LS: This patient was diagnosed as having phenylketonuria at ten months of age. The eyes were blue, the skin fair, and the hair blond.



Figure 15.5 BA and LA: Severely mentally impaired, institutionalized brothers with untreated phenylketonuria. They were quite fair of hair and skin.



Figure 15.6 AD: A Saudi Arabian infant with classic phenylketonuria. Routine neonatal screening had not yet been initiated in that country at the time of diagnosis.

the odor is most often noted in patients with disorders of urea cycle treated with sodium phenylacetate, and in these circumstances it may be pervasive.

Patients with PKU are often quite good-looking children. They are fair-haired, fair-skinned, and blue-eyed in over 90 percent of the cases (Figures 15.4 and 15.5) [4]. However, there is no amount of pigment in skin, hair, or irides that excludes the diagnosis. In a family, the pigmentation of the untreated affected child is less than that of unaffected members (Figure 15.7). The dermatitis is usually mild (Figure 15.3), and it is absent in three-quarters of the patients, but it may be a bothersome symptom.

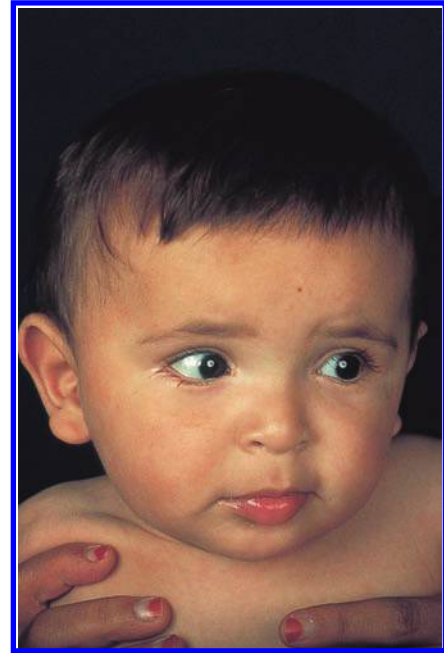


Figure 15.7 EQ: Another Saudi infant with classic phenylketonuria was considerably less pigmented than expected.



Figure 15.8 This ten-year-old boy was found in a Romanian orphanage. A diagnosis of phenylketonuria was made at the age of seven years. He had not been treated. He was very hyperactive and had seizures. His hair was blond, but his eyes hazel. He had hypertonia and had a rapid unusual limping gait, in which he leaned forward to the left, toe-walked, swinging his right arm, and keeping the left at his side.

Patients may complain of intractable itching in the absence of visible cutaneous lesions. Sclerodermatous skin has been reported [10] in an infant with PKU.

Neurological manifestations are not usually prominent, but about a third of the patients may have all of the signs of cerebral palsy [11]. They have spasticity and hypertonia, and have increased deep tendon reflexes. Only about 5 percent have these manifestations to a severe degree. They may have contractures and limitation of mobility. Hyperactivity is common (Figure 15.8), and there may be abnormalities of gait. Another one-third of the patients have very mild neurological signs, such as a unilateral Babinski response or hyperactive deep tendon reflexes. Another third of untreated patients have no neurological signs, except for mental impairment.

Seizures occur in about a quarter of the patients [7]. They are usually neither prominent nor difficult to manage. Nevertheless, about 80 percent have electroencephalograph (EEG) abnormalities [12]. Hyperactivity and behavior problems are common. Purposeless movements, rhythmic rocking, stereotypy, tremors, and athetosis may be seen. Somatic development tends to be normal, but stature may be short. Patients treated from the neonatal period are of normal stature. Some patients have minor malformations [13]. These include widely spaced incisor teeth, pes planus, partial syndactyly, and epicanthus (Figure 15.3). Congenital heart disease appears to be more common in PKU than in the general population [14]. Some patients have microcephaly [11]. It has been calculated that in the absence of treatment a patient may lose 50 IQ points in the first year of life [15]. In the past, a majority of patients with untreated PKU required institutional care (Figure 15.5).

GENETICS AND PATHOGENESIS

Phenylketonuria is transmitted by an autosomal recessive gene on chromosome 12q22-24.1. The gene for human PAH has been cloned. Hundreds of different alleles have been discovered, over 90 percent of which cause disease [16, 17], but only five are responsible for most human disease; the rest are rare. Almost two-thirds are missense mutations; 13 percent are small deletions; 12 percent splice site mutations; 6 percent nonsense mutations; and 1 percent small insertions. Large deletions are rare.

Restriction enzyme polymorphism permitted heterozygote detection and prenatal diagnosis in the approximately 75 percent of families in which relevant polymorphism was identified [18]. Affected fetuses have been diagnosed prenatally in this way. Restriction fragment length polymorphism (RFLP) exists in or near the phenylalanine hydroxylase gene that permits assessment of the transmission of alleles within a family. A composite family of RFLPs on an allele is referred to as an 'RFLP haplotype'. Some 50 haplotypes have been described for the phenylalanine hydroxylase locus. Once the mutation in the phenylalanine hydroxylase gene is known, mutational analysis may be used for prenatal diagnosis and for heterozygote detection. This provides a practical argument for seeking the precise

molecular diagnosis. In the best studied Northern European population, eight mutations have resulted in 64 percent of the mutant phenylalanine hydroxylase chromosomes [4, 5]. Two mutations, in each of which there was zero enzyme activity and cross-reacting material (CRM), accounted for 46 percent; these were an arginine to tryptophan change in amino acid residue 408 (R408W) of exon 12 and a splicing mutation of intron 12. A number of the abnormal alleles identified have involved cytosine-phosphate-guanine (CpG) dinucleotides, which are known to be highly mutable.

Expression of the mutant genes and assessment of enzyme activity *in vitro* has permitted correlations of phenotype with genotype [4]. Correlations with pretreatment concentrations of phenylalanine, and phenylalanine tolerance and the response to oral loading with protein were quite strong. The R408W mutation expressed 100 percent of mRNA, but less than 1 percent of enzyme activity and immunoreactive hydroxylase protein [4, 19]. Arginine to glutamine mutations in exons 5 and 7 were associated with variant phenotypes.

Different mutations have been found in other populations. Most of these have been missense mutations, such as the one that leads to complete inactivation of the enzyme in North African Jews [20]. A few deletions have been observed, such as the 22-bp deletion in exon 6 in an Arab family [21]. The primary effect of mutation in the gene is defective activity of the enzyme. This has been demonstrated by liver biopsy [22] in which activity correlated well with *in vitro* expression analysis of the mutant gene. Correlations were also excellent in eight patients tested *in vivo* with deuterated phenylalanine [23]. Analysis of genotype-phenotype correlations in an assembly of 365 patients reported [24] revealed a predominantly predictable or consistent phenotypic degree of severity in the majority. However, there were a number of genotypes that were associated with inconsistent phenotypes – both classic PKU and the variant hyperphenylalaninemia in patients with the same genotype. In a head-to-head comparison between a mutation 311C→A (A104D), associated with mild hyperphenylalaninemia, and 470G→A (R157N), with classic PKU, *in vitro* expression studies and *in vivo* ¹³C-phenylalanine metabolism [25] indicated quite different impacts of mutation on enzyme function and physical properties. The severe mutation coded for a protein that was degraded faster than the milder variant. Although many individuals have missense mutations in the PAH gene, how such a defective enzyme will function depends on protein folding. In this regard, several studies have been published on V_{\max} , K_M , and binding of its natural cofactor BH₄ [26, 27]. The results on more than 500 patients worldwide identified 60 mutant alleles. These studies clearly indicate the importance of genotyping PAH for management as well as for BH₄ treatment [27]. In 2004, Erlandsen *et al.* [28] showed that a subset of PKU patients who have a distinct genotype, showed normalization

of blood phenylalanine level upon administering its cofactor BH_4 . The relationship between PAH mutations and biochemical and metabolic phenotypes has also been reported [29]. The interaction of BH_4 and its impact on PAH protein folding is of major significance in the management of hyperphenylalaninemia with certain phenotypes of PAH [30]. BH_4 increases the activity of PAH by acting as a chemical chaperone preventing protein misfolding, thus protecting PAH from inactivation [31]. Sapropterin dihydrochloride (kuvan) which is the synthetic analog of the natural cofactor, 6R-isomer of tetrahydrobiopterin, has the same activation mechanism of PAH [32].

The incidence of classic PKU has become clear from experience with the screening programs around the world. The incidence in the United States is approximately 1:10,000. Approximately 1 in 50 is a carrier of the gene. Heterozygosity has been demonstrated by assay of the enzyme in liver, and of course, by mutational analysis.

Phenylalanine hydroxylase, the defective enzyme in PKU, has a tetrahydrobiopterin cofactor that is required for the hydroxylation of phenylalanine. In the hydroxylase reaction, a quinonoid dihydrobiopterin is formed. The reduction of this compound to reform tetrahydrobiopterin is catalyzed by dihydropteridine reductase [33, 34]. The quinonoid oxidation product is unstable and unless it is promptly reduced it forms the 7,8-dihydrobiopterin and is no longer a substrate for dihydropteridine reductase, but it can be reduced by dihydrofolate reductase in the presence of the reduced form of nicotinamide-adenosine dinucleotide phosphate (NADPH). The synthesis of biopterin begins with guanosine triphosphate and proceeds through reduced neopterin (α -D-erythro-7,8-dihydroneopterin triphosphate) to a dihydro-precursor of tetrahydrobiopterin [35, 36].

Three isozymes of phenylalanine hydroxylase have been found in liver [37]. The three isozymes have identical molecular weights and kinetic constants, but differ in charge [37, 38]. In classic PKU, all three isozymes are missing. Immunochemical study of phenylalanine hydroxylase from phenylketonuric human liver has revealed no cross-reacting material using antibody that reacted with normal hepatic enzyme [39]. The activity of phenylalanine hydroxylase in classic PKU has been reported as undetectable [3, 40–43].

In the presence of a defect in phenylalanine hydroxylase, the first compound that accumulates is phenylalanine itself. In classic PKU, the plasma concentration of phenylalanine is virtually always above $1200 \mu\text{mol/L}$. It is transaminated (Figure 15.1) to form phenylpyruvic acid, the phenylketone for which the disease was named. There is a roughly linear relationship between the concentrations of phenylalanine in the blood and the urinary excretion of phenylpyruvic acid [44]. This is the compound that is responsible for the positive ferric chloride (FeCl_3) test. A deep green color is seen on the addition of 10 percent (FeCl_3) to the urine of patients with untreated PKU (Figure 15.2). Phenylpyruvic

acid is subsequently converted to phenyllactic acid, phenylacetic acid, and phenylacetylglutamine. Phenylpyruvate is also hydroxylated in the ortho position, ultimately yielding orthohydroxyphenylacetic acid. These are not abnormal metabolites, but normal ones that occur in abnormal amounts in PKU. It is current theory that it is this abnormal chemical milieu in which the patient with PKU lives that produces the severely impaired mental development and other manifestations of the disease.

There are a variety of secondary effects of the accumulation of phenylalanine and its metabolites. Decreased pigmentation has been related to the inhibition of tyrosinase by phenylalanine. Decreased levels of 5-hydroxytryptamine (serotonin) appear to be due to inhibition of 5-hydroxytryptophan decarboxylase by phenylpyruvic, phenyllactic, and phenylacetic acids. Decreased amounts of epinephrine, norepinephrine, and dopamine are presumably caused by inhibition of dopamine decarboxylase. The metabolites that accumulate in PKU also inhibit glutamic acid decarboxylase in brain, and this would decrease levels of 4-aminobutyric acid (GABA). Studies of protein synthesis and turnover *in vivo* via continuous infusion of ^{13}C -leucine have revealed no abnormality in PKU [45].

DIAGNOSIS

The diagnosis of PKU should be made in the neonatal period. This is accomplished by the routine screening of all infants for an elevated concentration of phenylalanine in the blood. It is generally carried out on discharge from hospital after the initiation of protein-containing feedings. A drop of blood collected from the heel on filter paper is analyzed for phenylalanine by the bacterial inhibition method developed by Guthrie and Suzi [46], or by a quantitative determination of the concentration of phenylalanine. This is now incorporated into expanded programs of screening employing tandem mass spectrometry (MS/MS). A positive screening test is usually repeated. A second positive is followed up with quantitative assay of the concentrations of phenylalanine and tyrosine in the blood confirming the phenylalaninemia and excluding transient tyrosinemia of the newborn, a common cause of a positive screening test. In the presence of an elevated concentration of phenylalanine and normal or reduced tyrosine, the patient may be admitted to hospital, where protein and phenylalanine intake are carefully monitored and fresh urine specimens collected. Patients with classical PKU ingesting a normal diet display a very rapid rise of plasma phenylalanine to levels well over $1800 \mu\text{mol/L}$. A concentration of $1200 \mu\text{mol/L}$ or more is diagnostic of PKU. Patients with classic PKU also excrete the metabolites phenylpyruvic acid and orthohydroxyphenylacetic acid in the urine. Cofactor abnormalities (Chapter 21) can be ruled out at that time. Precise determination of the concentration of phenylalanine in blood is of major importance. In this regard, the use of Guthrie test is inappropriate, particularly

- When newborn screen positive: —————→ Obtain plasma for quantitative amino acids
Begin low phenylalanine diet
- Plasma phenylalanine over 340 $\mu\text{mol/L}$ (6 mg/dL). Plasma phenylalanine 150–300 $\mu\text{mol/L}$ (2.5–6 mg/dL)
Repeat ↓
Plasma phenylalanine > 180 $\mu\text{mol/L}$ ← elevated ← Repeat —————→ Normal level < 150 $\mu\text{mol/L}$
no further control
- Exclude cofactor deficiency
Urinary pterins

Dihydropteridine reductase ————— Abnormal —————→ CSF BH₄, neurotransmitters
Enzyme diagnosis
Treatment with BH₄, L-DOPA
5-OH tryptophan, carbidopa.
- Exclude transient tyrosinemia
Tyrosine concentrations high, exceed phenylalanine —————→ Continue to monitor concentrations and determine
transient status.
Consider ascorbic acid treatment to accelerate.
- Phenylalanine elevated; tyrosine low.
Phenylalanine > 600 $\mu\text{mol/L}$ (10 mg/dL) – Classic PKU —————→ Diet therapy.
Phenylalanine < 300 $\mu\text{mol/L}$ – Hyperphenylalaninemia —————→ Normal diet.
Continue to monitor phenylalanine.
Phenylalanine 300–600 $\mu\text{mol/L}$. Hyperphenylalaninemia —————→ Needs some dietary restriction.
- Initial Dietary Therapy for Classic PKU means delete phenylalanine from diet as follows (phenex-1 or Lofenelac 0.7–1.0 cal/mL).

Plasma Phenylalanine		Delete Phenylalanine for:	Monitor Plasma
($\mu\text{mol/L}$)	(mg/dL)	Hours	Quantative Phenylalanine*
240–605	(4–10)	24	qd
605–1210	(10–20)	48	qd
1210–2420	(20–40)	72	q1–3d
>2420	(>40)	96	q1–3d

*To prevent phenylalanine deficiency
When plasma phenylalanine reaches the treatment range phenylalanine is added to the diet

Individual amino acid requirements vary. The following are guidelines for initial dietary phenylalanine content dependent on the maximum pretreatment plasma levels:

Plasma Phenylalanine		Dietary Phenylalanine
($\mu\text{mol/L}$)	(mg/dL)	mg/kg
<605	(<10)	70
605–1210	(10–20)	55
1210–1815	(20–30)	45
1815–2420	(30–40)	35
>2420	(>40)	25

Monitor neonatal levels sufficiently frequently to establish a steady state concentration at the desired level while the infant receives a constant intake of phenylalanine and tyrosine.
Aim to keep plasma phenylalanine between 100 and 300 $\mu\text{mol/L}$.

- Monitor thereafter every week until 6 months old;
q 2 weeks until 1-year-old
q 4 weeks until 3-years-old
q 6 months until 12-years-old
Yearly thereafter.

in MS/MS-based screening the most common finding was 1/5882 hyperphenylalaninemia. It is interesting to note that while classic PKU + mild PKU were 20 patients, 40 were found to have mild hyperphenylalaninemia [47]. A protocol for the management of the newborn detected by a positive screening test is given in [Table 15.1](#).

The diagnosis of PKU is often challenged [48] with dietary phenylalanine 90–110 days after diagnosis and again after one year of age [15]. A conventional challenge in a three- to six-month-old infant is a 3-day intake of 24 oz of evaporated milk:water (1:1) which provides 180 mg/kg of phenylalanine. The challenge can be adjusted to 180 mg/kg of phenylalanine for an older, larger child. In most patients with classic PKU, the challenge yields a sharp rise in the plasma concentration of phenylalanine to 1800–2400 $\mu\text{mol/L}$ in 48 hours, at which time the challenge is stopped.

It is important to remember that this challenge was developed for use with infants, and the predominant experience is at the three-month level. A dose of 180 mg/kg per day of phenylalanine for 3 days would be a sizeable challenge for an older child or adult with PKU. In fact, symptomatic hypoglycemia and hyperinsulinemia have been reported in a 15-year-old patient so challenged [49]. Infants in whom this test did not yield levels higher than 1200 $\mu\text{mol/L}$ [42] were classified as variants. Currently, we consider those with levels over 600 $\mu\text{mol/L}$ as having classic PKU (Table 15.1).

It was the widespread screening of infant populations that led to the recognition that not all patients with hyperphenylalaninemia have classic PKU. Some variants represent molecular heterogeneity at the phenylalanine hydroxylase locus specifying variant enzymes with partial activity. Most of the variants have phenylalanine concentrations under 1200 $\mu\text{mol/L}$, and such infants can tolerate more than 75 mg/kg of phenylalanine per day. A small number of variant patients have been studied by liver biopsy [39, 42, 50], and in each a substantial defect in phenylalanine hydroxylase activity was demonstrated. Most have had levels of activity that were 10–20 percent of normal.

Transient phenylalaninemia may represent an isolated delay in the maturation of phenylalanine metabolizing enzymes. It is because of this phenomenon that patients with phenylalaninemia are routinely tested for their dietary tolerance to phenylalanine during the first year of life.

TREATMENT

The treatment of PKU is the provision of a diet sufficiently low in phenylalanine that the serum concentrations are maintained in a reasonable range and metabolites disappear from body fluids. This requires the provision of enough phenylalanine to meet the normal requirements of this essential amino acid for growth. It also requires the frequent quantitative assessment of the concentration of phenylalanine in the blood (Table 15.1). Levels recommended as acceptable have ranged from 180 to 900 $\mu\text{mol/L}$. However, Smith and colleagues [51] have recommended a smaller window between 120 and 300 $\mu\text{mol/L}$. Their data show a linear relationship between IQ and mean concentration during therapy over



Figure 15.9 A two-year-old Saudi phenylketonuria patient who was treated as a newborn and is now in normal school is shown with his older brother who also had phenylketonuria, but was detected at one year of age and had an IQ of approximately 70. Skin and hair color were dark in both.

300 $\mu\text{mol/L}$, but the differences were not clear until levels exceeded 800 $\mu\text{mol/L}$. Setting the lower level at 120 $\mu\text{mol/L}$ is less secure; patients with long periods below this level appeared to have low IQ levels, only in the early cohort born prior to 1971, and no other lower limit was assessed. We strive to keep levels below 300 $\mu\text{mol/L}$ and find that most patients in steady state do not approach any lower limit areas.

A patient detected in the neonatal period and managed with these guidelines should have an IQ in the normal range (Figure 15.9). The prevention of clinical disease by the restriction of dietary phenylalanine has provided the strongest evidence for the concept that the clinical manifestations of the disease result from the abnormal chemical milieu that follows the genetic defect. Preparations are now available that facilitate long-term treatment (Lofenalac-Mead-Johnson; Analog XP-Ross) and listings are available of the phenylalanine contents of foods and sources of low-protein products [52].

Sapropterin dihydrochloride (kuvan) which is the synthetic analog of the natural cofactor, 6R-isomer of tetrahydrobiopterin, is used as a chemical chaperone preventing protein misfolding thus protecting PAH from inactivation [32]. Its use is approved in the United States, European Union, and Japan. At a daily dose of 20 mg/kg, kuvan can result in increased dietary tolerance for phenylalanine or, in rare instances, replacement of the phenylalanine-restricted diet. It has the potential for the treatment of those with mild hyperphenylalaninemia who are not on diet, challenging neonates who have hyperphenylalaninemia identified by newborn screening, and the treatment of pregnant women with PKU [53, 54].

In the history of the management of the patient with PKU, the issue of termination of the diet has undergone

evolution. It was once thought that in most patients with PKU the diet could safely be stopped at five years of age. In a study from Poland, a decrease in IQ was found in most patients with classic PKU after discontinuing the diet [55]. Furthermore, there were difficulties in adaptation problems with performance in school and EEG abnormalities. Similarly, among 47 patients with PKU, treated at the Hospital for Sick Children in London, given a normal diet between the ages of five and 15, there was a statistically significant fall in IQ of 5–9 points after discontinuing treatment [56]. The change in IQ was uniformly negative and was progressive. Among 21 patients treated at the Universitäts-Kinderklinik, Heidelberg, who were given a relaxed low phenylalanine diet rather than a normal diet at about the same time as the London group, there were smaller and nonsignificant falls in IQ. Data from the United States Collaborative Study on 115 children suggested that discontinuing dietary treatment at six years of age led to a reduction in IQ [57]. There were significant differences in school performance as measured by the Wide Range Achievement Test.

Actually, the IQ alone may not be the most sensitive criterion on which to base this decision in an individual patient. Other aspects of clinical condition which might benefit from treatment longer than necessary to produce a stable IQ might affect the way a child functions in society. Behavioral abnormalities are common in children with PKU, despite early diagnosis and treatment and normal IQ. Mannerisms, hyperactivity, and signs of anxiety have been reported in eight-year-old children treated since early neonatal diagnosis, and those whose diet was less strictly controlled were twice as likely to display abnormal behaviors than those more strictly controlled [58]. On the other hand, a study [59] of 586 German ten-year-old PKU patients via a personality questionnaire failed to reveal differences from controls. The long-term effect of treatment on intelligence of patients with PKU has been assessed in a British study of 15 well-managed individuals with PKU treated by two years of age, who had normal IQ. They were found to have defects in planning and attention domains [60]. A comparative study on 35 well-managed PKU patients as compared to 35 diabetic individuals matched in sex, age, and socioeconomic status were observed to have a reduced speed in performance [61]. It is certain that early detection and treatment prevent serious consequences of PKU. However, more and more hidden disabilities are being noticed later in life in well-treated PKU patients. These include mostly executive dysfunction (EF), mild reduction in mental processing speed, social difficulties, and difficulties in establishing interpersonal relationships and emotional problems, which may go unnoticed if not particularly looked for [62]. Children and adolescents with PKU showed lower performance in several EF skills: initiation of problem solving, concept formation, and reasoning. Performance on EF tasks requiring inhibitory control, cognitive flexibility, and set shifting decreased at higher levels of phenylalanine. Levels of phenylalanine

were positively correlated to age and inversely related to dietary adherence. Therefore, there is a need to monitor EF skills in patients with PKU during the transition to, and during, adolescence [63].

A study in 2010 on well-treated PKU patients detected suboptimal outcomes in neurocognitive/psychosocial outcome, in nutrition/growth, quality of life, in bone and brain pathology, and in incidence of maternal PKU [64]. The exact biochemical mechanism underlying the subtle neurocognitive defects remains unknown. However, ^{18}F -deoxyglucose positron emission tomography (PET) revealed decreased glucose utilization in prefrontal cortex, somatosensory, and visual cortices, but increased activity in subcortical regions, such as the thalamus and limbic system [65]. Best management to prevent later appearance of subtle neurological defects remains early detection and vigorous treatment.

If high levels of phenylalanine continue to impair myelination, one might even expect treatment to be useful well up to puberty, since myelination at least in the formatio reticularis is not finished at eight years of life. In addition, the effect of high concentrations of phenylalanine on synaptogenesis is not known. Some older patients find that their skin feels better with modest restriction of phenylalanine. In any case, the rigidity with which one controls the level of phenylalanine can probably be relaxed after six years of age, but it is prudent to continue some restriction in the intake of phenylalanine. A study of 25 adults with PKU all of whom had been treated early [66] revealed normal intelligence, but in each patient scores were lower than control siblings in measures of intelligence and attention. Patients were advised to continue dietary restriction for life, but only ten followed this regimen. Others discontinued treatment before or during adolescence. Intellectual outcome appears to have best been predicted by the presence or absence of early insult to brain, while performance on a test of problem-solving correlated best with concurrent levels of phenylalanine even in adulthood. As patients become older, some relaxation of dietary control appears inevitable. In a recent study of 95 patients treated from the neonatal period and assessed at 12 years of age, best cognition results were those of patients whose phenylalanine values were kept consistently below $900\text{ }\mu\text{M}$.

Reduced concentrations of carnitine in serum have been found in patients with PKU [67]. This was the case in those less than two years of age managed with a restricted diet. In contrast, untreated infants with PKU had normal levels of carnitine as did older patients. The data provide an argument for supplementation with carnitine at least in infants treated for PKU.

Poor linear growth has been observed in some patients with PKU, and this has been thought to result from protein insufficiency. The level of prealbumin has been found [68] to correlate well with protein adequacy in this disease, and the threshold level is 20 mg/dL . Linear growth can be expected to be impaired in patients with levels lower than 20 mg/dL .

MATERNAL PKU

Elevated phenylalanine blood levels in pregnant women cause a syndrome known as 'maternal PKU' (MPKU). The elevated phenylalanine has teratogenic effects on the developing fetus. The findings include global developmental delay, microcephaly, facial dysmorphism, congenital heart disease (CHD), and low birth weight [69]. These findings are dramatically reduced when maternal phenylalanine levels are well maintained between 120 and 360 μM . Other studies as well have indicated that if the metabolic control is started 8 weeks before pregnancy, a better result is obtained. A British study on all MPKU between 1978 and 1997 compared head circumference, IQ at four and eight years of age, and CHD. Abnormalities were significantly lower when the treatment of the mother was initiated before 8 weeks of pregnancy [70]. Education of young women with PKU and initiation of metabolic control at least 8 weeks before pregnancy is essential for the prevention of MPKU [70, 71].

Male patients with PKU may have low sperm counts and semen volume. A survey of male patients over 18 years in the United States identified 40 men who had 64 children, but did not yield data on fertility rate [72]. Abnormalities were not identified in live-born offspring that could be related to paternal PKU.

REFERENCES

1. Folling A. Über Ausscheidung von Phenylbrenztraubensäure in den Harn als Stoffwechselanomalie in Verbindung mit Imbezillität Hoppe-Seyler's. *Z Physiol Chem* 1934; **227**: 169.
2. Penrose L, Quastel JH. Metabolic studies in phenylketonuria. *J Biochem* 1937; **31**: 266.
3. Jervis GA. Phenylpyruvic oligophrenia deficiency of phenylalanine-oxidizing system. *Proc Soc Exp Biol Med* 1953; **82**: 514.
4. Okano Y, Eisensmith RC, Butler F *et al*. Molecular basis of phenotypic heterogeneity in phenylketonuria. *N Engl J Med* 1991; **324**: 1232.
5. Scriver CR. Phenylketonuria – genotypes and phenotypes. *N Engl J Med* 1991; **324**: 1280.
6. Jervis GA. Phenylpyruvic oligophrenia. *Assoc Res Nerv Ment Dis Proc* 1954; **33**: 259.
7. Pitt DB, Dansk DM. The natural history of untreated phenylketonuria. *J Pediatr Child Health* 1991; **27**: 189.
8. Partington MW. The early symptoms of phenylketonuria. *Pediatrics* 1961; **27**: 465.
9. Centerwall W. Phenylketonuria. *Med Bull Los Angeles Child Hosp* 1959; **63**: 83.
10. Haktan M, Aydin A, Bahat H *et al*. Progressive systemic scleroderma in an infant with partial phenylketonuria. *J Inher Metab Dis* 1989; **12**: 486.
11. Paine RS. The variability in manifestations of untreated patients with phenylketonuria (phenylpyruvic aciduria). *Pediatrics* 1957; **20**: 290.
12. Low NW, Bosma JF, Armstrong MD. Studies on phenylketonuria. *Arch Neurol Psychiatr* 1957; **77**: 359.
13. Cowie V. Phenylpyruvic oligophrenia. *J Mental Sci* 1951; **97**: 505.
14. Verkerk PH, Van Spronsen FJ, Smith GPA *et al*. Prevalence of congenital heart disease in patients with phenylketonuria. *J Pediatr* 1991; **119**: 282.
15. Koch R, Blaskovics M, Wenz E *et al*. Phenylalaninemia and phenylketonuria. In: Nyhan WL (ed.). *Heritable Disorders of Amino Acid Metabolism*. New York: John Wiley & Sons, 1974: 109.
16. Scriver CR, Byck S, Prevost L *et al*. The phenylalanine hydroxylase locus: a marker for the history of phenylketonuria and human genetic diversity. *Ciba Found Symp* 1996; **197**: 73.
17. Scriver CR, Waters PJ, Sarkisian C *et al*. PAHdb: A locus-specific knowledge base. *Hum Mutat* 2000; **15**: 99.
18. Woo SLC, Lidsky AS, Guttler F *et al*. Cloned human phenylalanine hydroxylase gene allows prenatal diagnosis and carrier detection of classical phenylketonuria. *Nature* 1983; **306**: 152.
19. Svensson E, Eisensmith RC, Dworniczak B *et al*. Two missense mutations causing mild hyperphenylalaninemia associated with DNA haplotype 12. *Hum Mutat* 1992; **1**: 129.
20. Weinstein M, Eisensmith RC, Abadia V *et al*. A missense mutation S349P completely inactivates phenylalanine hydroxylase in North Africa Jews with phenylketonuria. *Hum Genet* 1993; **90**: 545.
21. Kleiman S, Schwartz G, Woo SLC, Shiloh Y. 122-bp deletion in the phenylalanine hydroxylase gene causing phenylketonuria in an Arab family. *Hum Mutat* 1992; **1**: 344.
22. Lyonnet S, Caillaud C, Rey F *et al*. Molecular genetics of phenylketonuria in Mediterranean countries: a mutation associated with partial phenylalanine hydroxylase deficiency. *Am J Hum Genet* 1989; **44**: 511.
23. Trefz RK, Erlenmaier T, Hunneman DH *et al*. Sensitive *in vivo* assay of the phenylalanine hydroxylating system with a small intravenous dose of heptadecuto L-phenylalanine using high pressure liquid chromatography and capillary gas chromatography/mass fragmentography. *Clin Chim Acta* 1979; **99**: 211.
24. Kayaalp E, Treacy E, Waters PJ *et al*. Human phenylalanine hydroxylase mutations and hyperphenylalaninemia phenotypes: a metaanalysis of genotype-phenotype correlations. *Am J Hum Genet* 1997; **61**: 1309.
25. Waters PJ, Parniak MA, Hewson AS, Scriver CR. Alterations in protein aggregation and degradation due to mild and severe missense mutations (A104D R157N) in the human phenylalanine hydroxylase gene (PAH). *Hum Mutat* 1998; **12**: 344.
26. Gersting SW, Staudigl M, Truger MS. Activation of phenylalanine hydroxylase induces positive cooperativity towards the enzyme's natural cofactor. *J Biol Chem* 2010; **285**: 30868.
27. Karacić I, Meili D, Sarnavka V. Genotype-predicted tetrahydrobiopterin (BH4)-responsiveness and molecular genetics in Croatian patients with phenylalanine hydroxylase (PAH) deficiency. *Mol Genet Metab* 2009; **97**: 165–71.

28. Erlandsen H, Pey AL, Gámez A *et al.* Correction of kinetic and stability defects by tetrahydrobiopterin in phenylketonuria patients with certain phenylalanine hydroxylase mutations. *Proc Natl Acad Sci USA* 2004; **101**: 16903–8.
29. Kasnauskienė J, Cimbalistienė L, Kucinskas V. Predicting a clinical/biochemical phenotype for PKU/MHP patients with PAH gene mutations. *Genetika* 2008; **44**: 1397–403.
30. Dobrowolski SF, Pey AL, Koch R. Biochemical characterization of mutant phenylalanine hydroxylase enzymes and correlation with clinical presentation in hyperphenylalaninaemic patients. *J Inherit Metab Dis* 2009; **32**: 10–21.
31. Pey AL, Pérez B, Desviat LR *et al.* Mechanisms underlying responsiveness to tetrahydrobiopterin in mild phenylketonuria mutations. *Hum Mutat* 2004; **24**: 388–99.
32. Sanford M, Keating GM. Sapropterin: a review of its use in the treatment of primary hyperphenylalaninaemia. *Drugs* 2009; **69**: 461–76.
33. Kaufman S. Metabolism of phenylalanine hydroxylation cofactor. *J Biol Chem* 1967; **242**: 3934.
34. Craine JE, Hall ES, Kaufman S. The isolation and characterization of dihydropteridine reductase from sheep liver. *J Biol Chem* 1972; **247**: 6082.
35. Eto I, Fukushima K, Shiota T. Enzymatic synthesis of biopterin from D-erythrodihydroneopterin triphosphate by extracts of kidneys from Syrian golden hamsters. *J Biol Chem* 1976; **251**: 6505.
36. Gal EM, Nelson JM, Sherman AD. Biopterin: III. Purification and characterization of enzymes involved in the cerebral synthesis of 78-dihydrobiopterin. *Neurochem Res* 1978; **3**: 69.
37. Barranger JA, Geiger PJ, Huzino A, Bessman SP. Isozymes of phenylalanine hydroxylase. *Science* 1972; **175**: 903.
38. Tourian A. The unique identity of rat hepatoma phenylalanine hydroxylase. *Biochem Biophys Res Commun* 1976; **68**: 51.
39. Friedman PA, Kaufman A, Kang ES. Nature of the molecular defect in phenylketonuria and hyperphenylalaninemia. *Nature* 1972; **240**: 157.
40. Bartholome K, Lutz P, Bickel H. Determination of phenylalanine hydroxylase activity in patients with phenylketonuria and hyperphenylalaninemia. *Pediatr Res* 1975; **9**: 899.
41. Embden G, Baldes K. Über den Abbau des Phenylalanins im tierischen Organismus. *Biochem Z* 1913; **55**: 301.
42. Justice P, O'Flynn ME, Hsia DYY. Phenylalanine hydroxylase activity in hyperphenylalaninemia. *Lancet* 1967; **1**: 928.
43. Mitoma C, Auld RM, Udenfriend S. On the nature of enzymatic defect in phenylpyruvic oligophrenia. *Proc Soc Exp Biol Med* 1957; **94**: 634.
44. Armstrong MD, Low NL. Phenylketonuria: VII. Relation between age serum phenylalanine level and phenylpyruvic acid excretion. *Proc Soc Exp Biol Med* 1957; **94**: 142.
45. Thompson GN, Pacy PJ, Watts RWE, Halliday D. Protein metabolism in phenylketonuria and Lesch-Nyhan syndrome. *Pediatr Res* 1990; **28**: 240.
46. Guthrie R, Suzi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics* 1963; **12**: 338.
47. Niu DM, Chien YH, Chiang CC. Nationwide survey of extended newborn screening by tandem mass spectrometry in Taiwan. *J Inherit Metab Dis* 2010; **33**(Suppl. 2): S295.
48. O'Flynn ME, Holtzman NA, Blaskovics M *et al.* The diagnosis of phenylketonuria. A report from the Collaborative Study of Children Treated for Phenylketonuria. *Am J Dis Child* 1980; **134**: 769.
49. Ziyai F, Wong PWK, Justice P, Michals K. Protein-induced hypoglycemia in a phenylketonuric patient. *J Pediatr* 1978; **92**: 681.
50. Kang ES, Kaufman S, Gerald PS. Clinical and biochemical observations of patients with atypical phenylketonuria. *Pediatrics* 1970; **45**: 83.
51. Smith I, Beasley MG, Ades AE. Intelligence and quality of dietary treatment in phenylketonuria. *Arch Dis Child* 1990; **65**: 472.
52. Acosta PB, Schaeffler GE, Wenz E, Koch R. *PKU – A Guide to Management*. Berkeley, CA: California State Department of Public Health, 1972.
53. Levy H, Burton B, Cederbaum S, Scriver C. Recommendations for evaluation of responsiveness to tetrahydrobiopterin (BH(4)) in phenylketonuria and its use in treatment. *Mol Genet Metab* 2007; **92**: 287–9.
54. Trefz FK, Belanger-Quintana A. Sapropterin dihydrochloride: a new drug and a new concept in the management of phenylketonuria. *Drugs Today* 2010; **46**: 589–600.
55. Cabalska B, Duczynska N, Brozymowska J *et al.* Termination of dietary treatment of phenylketonuria. *Eur J Pediatr* 1977; **126**: 253.
56. Smith I, Lobascher ME, Stevenson JE *et al.* Effect of stopping low-phenylalanine diet on intellectual progress of children with phenylketonuria. *Br Med J* 1978; **2**: 723.
57. Koch R, Azen CG, Friedman EG, Williamson ML. Preliminary report on the effects of diet discontinuation in PKU. *J Pediatr* 1982; **100**: 870.
58. Smith I, Beasley MG, Wolff OH, Ades AE. Behavior disturbance in 8-year-old children with early treated phenylketonuria. *J Pediatr* 1988; **112**: 403.
59. Weglage J, Rupp A, Schmidt E. Personality characteristics in patients with phenylketonuria treated early. *Pediatr Res* 1994; **35**: 6.
60. Azadi B, Seddigh A, Tehrani-Doost M *et al.* Executive dysfunction in treated phenylketonuric patients. *Eur Child Adolesc Psych* 2009; **18**: 360–8.
61. Feldmann R, Denecke J, Grenzebach M, Weglage J. Frontal lobe-dependent functions in treated phenylketonuria: blood phenylalanine concentrations and long-term deficits in adolescents and young adults. *J Inherit Metab Dis* 2005; **28**: 445–55.
62. Gentile JK, Ten Hoedt AE, Bosch AM. Psychosocial aspects of PKU: hidden disabilities – a review. *Mol Genet Metab* 2010; **99**(Suppl. 1): S64–7.
63. Van Zutphen KH, Packman W, Sporri L *et al.* Executive functioning in children and adolescents with phenylketonuria. *Clin Genet* 2007; **72**: 13–18.
64. Enns GM, Koch R, Brumm V *et al.* Suboptimal outcomes in patients with PKU treated early with diet alone: revisiting the evidence. *Mol Genet Metab* 2010; **101**: 99.

65. Wasserstein MP, Snyderman SE, Sansaricq C, Buchsbaum MS. Cerebral glucose metabolism in adults with early treated classic phenylketonuria. *Mol Genet Metab* 2006; **87**: 272–7.
66. Ris MD, Williams SE, Hunt MM *et al.* Early-treated phenylketonuria: adult neuropsychological outcome. *J Pediatr* 1994; **124**: 388.
67. Vilaseca MA, Briones P, Ferre I *et al.* Controlled diet in phenylketonuria may cause carnitine deficiency. *J Inherit Metab Dis* 1994; **16**: 101.
68. Arnold GL, Vladutiu CJ, Kirby RS *et al.* Protein insufficiency and linear growth restriction in phenylketonuria. *J Pediatr* 2002; **141**: 243.
69. Koch R, Trefz F, Waisbren S. Psychosocial issues and outcomes in maternal PKU. *Mol Genet Metab* 2010; **99**(Suppl. 1): S68–74.
70. Lee PJ, Ridout D, Walter JH, Cockburn F. Maternal phenylketonuria: report from the United Kingdom Registry 1978–97. *Arch Dis Child* 2005; **90**: 143–6.
71. NIH and National Institute of Child Health and Human Development. Report of the NIH Consensus Development Conference on Phenylketonuria (PKU): screening and management. Bethesda, MD: The Institutes, 2001.
72. Fisch RO, Matalon R, Weisberg S, Michals K. Children of fathers with phenylketonuria: an international survey. *J Pediatr* 1991; **118**: 739.

Hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin

Introduction	123	Diagnosis	130
Clinical abnormalities	125	Treatment	130
Genetics and pathogenesis	128	References	131

MAJOR PHENOTYPIC EXPRESSION

Mental impairment, muscular rigidity, dystonic movements, myoclonic seizures, drooling, microcephaly, hyperphenylalaninemia, and defective synthesis of tetrahydrobiopterin (BH_4) because of defective activity of GTP cyclohydrolase, or 6-pyruoyltetrahydropterin synthase (PTPS), and defective recycling of BH_4 due to deficiency of dihydropteridine reductase (DHPR) and pterin-4 α -carbinolamine dehydratase (PCD).

INTRODUCTION

The existence of variant forms of hyperphenylalaninemia resulting from abnormalities of cofactor synthesis was predicted with the discovery of biopterin (Figure 16.1) and its role in the phenylalanine hydroxylase reaction (Figure 16.2) [1–4]. BH_4 is an essential cofactor not only for phenylalanine hydroxylase, but also for tyrosine and two tryptophan hydroxylases, three nitric oxide synthases, and glyceryl-ether monooxygenase. Defective activity of tyrosine and tryptophan hydroxylases is relevant to neurological deterioration in patients with BH_4 deficiency [5]. The first patients were reported in the 1970s as an outgrowth of the programs of neonatal screening for phenylketonuria (PKU). A majority of the patients recognized early were diagnosed because they developed

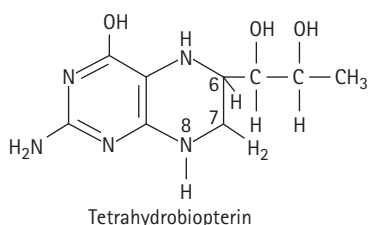


Figure 16.1 Tetrahydrobiopterin (BH_4), the pteridine cofactor of phenylalanine hydroxylase.

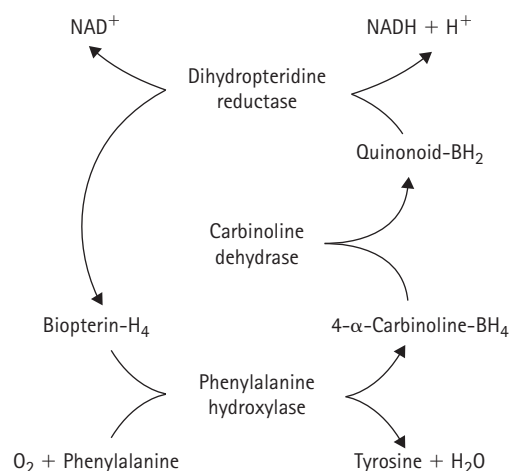


Figure 16.2 Dihydropteridine reductase and pterin- α -4carbinoline dehydratase, enzymes involved in regeneration of BH_4 following the phenylalanine hydroxylase reaction. Defects in this system lead to defective function of BH_4 in the metabolism of phenylalanine and the neurotransmitters. BH_4 , tetrahydrobiopterin; BH_2 , dihydrobiopterin; NAD1, nicotinamide dinucleotide, and NADH, its reduced form.

progressive cerebral deterioration despite an early neonatal diagnosis of hyperphenylalaninemia and effective dietary control of the levels of phenylalanine in blood.

Patients are now being diagnosed because of the initiation of programs in which all hyperphenylalaninemic infants are being investigated for the possibility of defective metabolism of bipterin. However, it has already been documented that it is possible to miss a patient with abnormal synthesis of BH_4 because early phenylalanine levels may be normal. Therefore, evaluation for a disorder in this pathway should be undertaken in infants with unexplained neurological disease. Five disorders are considered in this chapter: deficiencies of GTP cyclohydrolase I, 6-pyruvoyltetrahydropterin synthase, sepiapterin reductase, dihydropteridine reductase, and pterin-4- α -carbinolamine dehydratase (Figure 16.3). The clinical manifestations of all of them are quite similar, but the carbinolamine hydratase is relatively benign. Elevated phenylalanine would initiate investigation in most of these disorders. Sepiapterin reductase and the dominant form of GTP cyclohydrolase I deficiency are exceptions in which bipterin is deficient only in the brain [6]. The next step in elucidating a diagnosis is measurement of pterin metabolites in urine, and dihydropteridine reductase activity in blood spots. Enzyme activity may be assessed in erythrocytes or cultured fibroblasts. Diagnosis may also be secured by determination of mutations of the relevant gene. Improved prognosis with therapy makes prompt diagnosis and the timely initiation of therapy important.

In 1974, Bartholome [2] described a patient who was later found to have a block in the synthesis of BH_4 . This patient was initially diagnosed as having PKU, but had a progressive deterioration, although dietary restriction of phenylalanine had been exemplary. In the same year, Smith [3] described patients in whom their phenylalaninemia was atypical in that they had a progressive neurological illness despite restriction of the intake of phenylalanine. She postulated a disorder in BH_4 metabolism. In the same year, Kaufman and colleagues [4] reported deficiency of dihydropteridine reductase in such a patient.

Phenylalanine hydroxylase requires BH_4 for activity in the hydroxylation to tyrosine (Figure 16.2) [1, 2, 7]. In the conversion of phenylalanine to tyrosine, BH_4 is oxidized to its hydroxyl compound, 4 α -carbinolamine. This is recycled to quinonoid dihydropterin in a reaction catalyzed by 4 α -carbinolamine dehydratase (PCD) (EC 4.2.1.96) [6].

The oxidized quinonoid dihydropterin compound must be reduced to form BH_4 before it can again be active as a cofactor (Figure 16.2). The reduction is catalyzed by dihydropteridine reductase (DHPR) (EC 1.6.99.7) [4, 7–9]. The quinonoid oxidation product is unstable and, unless it is promptly reduced, it forms a 7,8-dihydrobiopterin, which is no longer a substrate for dihydropteridine reductase.

The synthesis of the tetrahydrobiopterin cofactor is originally from guanosine triphosphate (GTP), and it proceeds through a number of steps in which reduced neopterin triphosphate (7,8-dihydroneopterin triphosphate) is an intermediate (Figure 16.3) [10–12]. The first step is the GTP cyclohydrolase I reaction (GTPCH) (EC 3.5.4.16) [13]. The next step is the 6-pyruvoyltetrahydropterin synthase (6-PTS) (EC 4.6.1.10) [14, 15]. Sepiapterin reductase and the reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH) are involved in the conversion of 6PT to BH_4 . Aldose reductase and carbonyl reductases may also serve to catalyze this reaction, and this may be why defects in sepiapterin reductase have not been identified as causative of human disease.

The gene has been cloned for DHPR [16, 17]. The gene maps to chromosome 4 (p15.3). The gene for GTPCH is on chromosome 14q22.1–22.2. The cDNA for the human enzyme has been cloned [18]. The cDNA for 6-PTS has been cloned [19, 20] and mapped to chromosome 11q22.2–23.3 [21]. The gene for PCD has been localized to chromosome 10q22 [22]. Mutations have been identified in the genes for each of the four enzymes defective in human metabolism: DHPR [22], PCD [23, 24], GTPCH [25, 26],

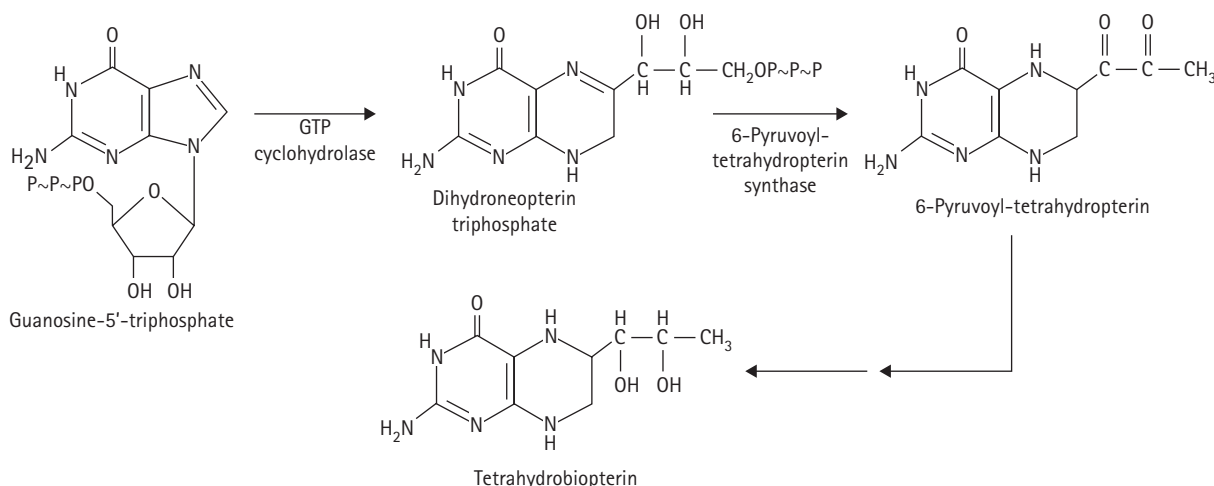


Figure 16.3 Bipterin synthesis; GTP cyclohydrolase I and 6-pyruvoyltetrahydropterin synthase are sites of clinically defective bipterin synthesis.

and 6-PTS [20]. These different abnormalities of bipterin metabolism account for less than 2 percent of patients with hyperphenylalaninemia. Each is inherited in an autosomal recessive fashion.

An international database [27] includes over 300 patients; over 200 had 6-PTS deficiency, over 100 DHPR deficiency, and fewer than 20 PCD deficiency, and 11 have GTPCH deficiency. Some remain unclassified, indicating the possibility of disorders yet to be identified. In addition to these disorders, there are a number of disorders of bipterin metabolism in which hyperphenylalaninemia does not occur, such as dihydroxyphenylalanine (DOPA)-responsive dystonia or Sagawa disease, which results from autosomal dominant mutations in GTPCH [28].

The spectrum of mutations in these pathways has been summarized by Thony and Blau [29]. Mutations of GCH1 were widely distributed. Only five out of 104 mutant alleles were found in the autosomal recessive form hyperphenylalaninemia with neurotransmitter deficiencies, while all the other mutants were found in patients with DOPA-responsive dystonia (Segawa disease) expressed as a dominant. Mutations in PTS amounted to 44 alleles scattered widely over the gene. Mutations in PCBD comprised nine mutant alleles, while in QDPR there were 29 mutant alleles. Seven different mutant alleles were found in the *SPR* gene.

CLINICAL ABNORMALITIES

The clinical manifestations in most of these disorders are indistinguishable except for PCD deficiency. These patients have a much milder phenotype [24]. They might have been categorized clinically as having mild hyperphenylalaninemia. They were first recognized on the basis of a high urinary neopterin and an unknown compound, which proved to be primapterine, a 7-isomer of bipterin the side chain of which is in the 6 position (Figure 16.1) [30–32].

The classic presentation of abnormality in BH₄ metabolism is of an infant who appears normal at birth, but is found on screening to have an elevated concentration of phenylalanine in blood. A tendency to low birth weight has been observed in patients with defective synthesis, especially with 6-PTS deficiency, but not in reductase-deficient patients. Failure to thrive may be impressive. Mild hypotonia may be present early. Some patients have had increased tone early [33], or there may be hypotonia of the trunk and hypertonia of the limbs. Development may be normal for two to three months; thereafter, a decrease in activity or a loss of head control may herald the onset of a progressive neurological degenerative disease (Figures 16.4, 16.5, 16.6, 16.7, 16.8, 16.9, and 16.10) [34, 35]. Onset may be with convulsions as early as three months of age [36].

Ultimately, these patients develop hypertonia, especially in the lower extremities (Figures 16.11 and 16.12) [36, 37]. There may be bradykinesia, episodic ‘lead pipe’



Figure 16.4 GH: A three-year-old girl with defective synthesis of bipterin. The diagnosis was made and bipterin replacement was begun along with 5-hydroxytryptophan, L-DOPA, and carbidopa treatments. Nevertheless, she was significantly neurologically impaired. She could sit unassisted and crawl. Muscle tone was decreased and deep tendon reflexes exaggerated. By six years of age, she had a wide-based ataxic gait and drooled frequently.



Figure 16.5 RM: A severely affected infant with 6-pyruvoyltetrahydropterin synthase (6-GPTS) deficiency. He had bradykinesia, rigidity, and myoclonus. Color of the skin and eyes were fair. At the age of nine, after treatment with BH₄ and biogenic amine precursors, he attended normal school and had average performance for age and grade.

rigidity, or ‘cog-wheel’ rigidity [35]. The picture may be reminiscent of Parkinson disease. A ‘stiff baby’ syndrome has been described [38] in which torticollis was present and progressive rigidity. Episodes of extensor posturing of the extremities and opisthotonic arching of the back are characteristic. The hands are pronated. Deep tendon reflexes are increased. Clonus is frequently elicited and the Babinski responses are present [39]. Drooling is a function of difficulty in swallowing and handling secretions. Feeding becomes difficult as well. The patient may be unable to swallow even puréed foods [33]. The typical patient is

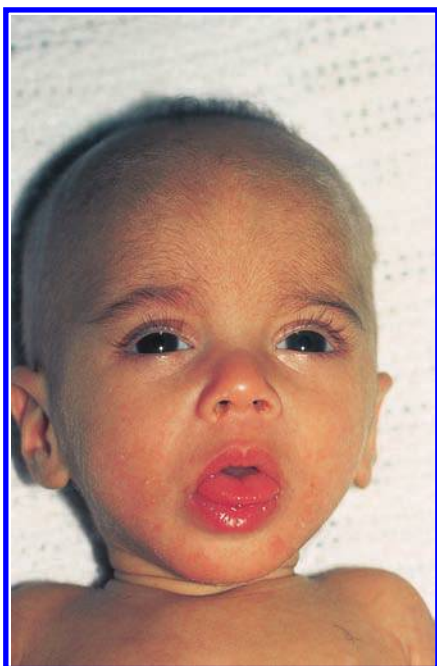


Figure 16.6 Close-up of the face of RM.



Figure 16.8 AM: Another patient with 6PTS deficiency, illustrating the hypotonia. Despite late treatment, he achieved some milestones.



Figure 16.7 RM: On the right, 3.5 years later; next to him, his sister who was diagnosed and treated early, was normal neurologically with a normal IQ for age.

withdrawn and appears drowsy or expressionless, but irritability is also characteristic. Involuntary movements may be dystonic in nature. There may be oculogyric spasms. Some patients have tremors. Seizures are characteristically myoclonic and myoclonic seizures may be the presenting complaint [35], but grand mal seizures may occur as well. The electroencephalograph (EEG) pattern is abnormal.

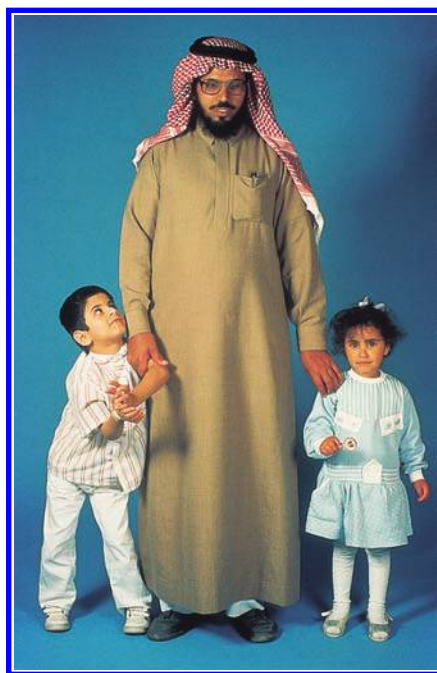


Figure 16.9 AM: Two years later, illustrating his ability to stand with a broad gait and posturing. The sister on the left, also affected, appeared normal, having been diagnosed and treated early.

The impairment in intellectual function is usually profound, but there is heterogeneity, and some patients with only mildly impaired mental development have been described. These have tended to be younger patients, and

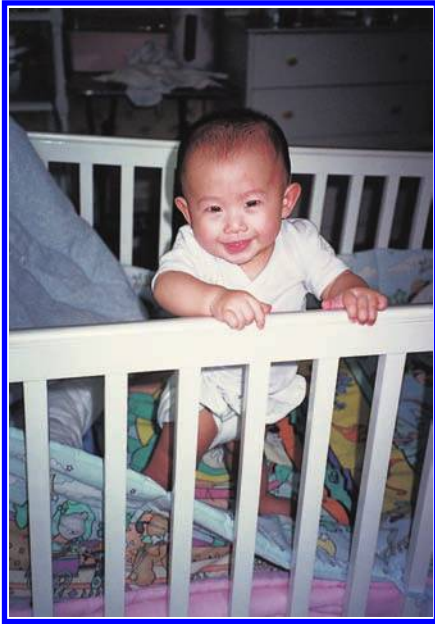


Figure 16.10 IT: An infant with defective BH_4 synthesis detected by newborn screening and treated early was developing well.



Figure 16.12 The sister of the patient in Figure 16.11 was diagnosed as having spasticity and impaired mental development at four years of age. At six years of age, she died of pneumonia.



Figure 16.11 A four-month-old Saudi patient with PTPS deficiency who was diagnosed in the newborn period and treated with BH_4 , DOPA, and 5-hydroxytryptophan. She has had normal growth and development.

progressive deterioration of the IQ has been documented, for instance from 83 at 20 months to 24 at 11 years of age [36]. Microcephaly may be a consequence, and computed tomography (CT) scan or magnetic resonance imaging (MRI) of brain may reveal cerebral atrophy [40] or lucency

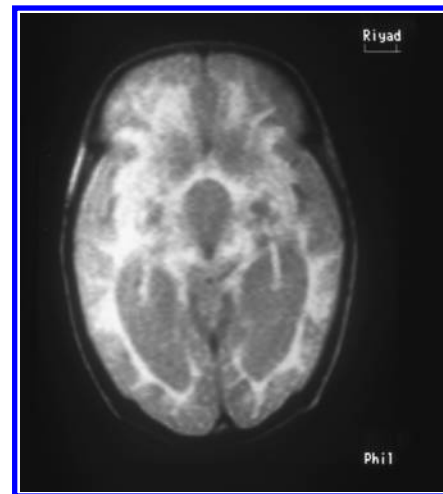


Figure 16.13 Magnetic resonance image of a patient with PTPS deficiency. There was increased T_1 signal intensity in the frontal lobe.

of the white matter [41] (Figure 16.13). Abnormalities of the basal ganglia have been described and a pattern of intracranial calcification similar to that of methotrexate toxicity or folate malabsorption [42, 43].

Patients not treated, in whom levels of phenylalanine are not kept from being elevated, may develop the fair hair and skin or relative lack of pigmentation that is characteristic of the patient with PKU [35–39]. Some patients have had episodes of hyperthermia without apparent infection [39]. Severe bronchopneumonia may require intensive care [35].

Death occurs often within the first five years of life [44]. One patient died of sudden infant death syndrome (SIDS) in hospital [35].

Patients with defective metabolism of BH_4 usually have hyperphenylalaninemia. Most are detected initially in programs of neonatal screening for PKU. However, some patients have had levels more consistent with atypical hyperphenylalaninemia or the variant forms of defective phenylalanine hydroxylase, and at least one patient in whom hyperphenylalaninemia has not been observed has been discovered through the study of siblings of known patients with BH_4 defects [45].

Dominantly inherited GTP-cyclohydrolase deficiency, Segawa disease, is also known as DOPA-responsive dystonia. The disease manifests classically with difficulty walking as a result of dystonia. There may be parkinsonian features involving reduced facial expression and slowed movements of fingers [46–48]. Abnormal sleep includes sleeping and nightmares.

DOPA-responsive dystonia, like sepiapterin reductase deficiency, does not manifest hyperphenylalaninemia [49]. The disorders are usually diagnosed on the basis of low levels of the neurotransmitter homovanillic acid and reduced levels of neopterin and BH_4 in the cerebrospinal fluid. DOPA-responsive dystonia is eminently treatable with L-DOPA, therefore its recognition is very important.

Hyperphenylalaninemia in the neonatal period has been reported [50] in sepiapterin reductase-deficient patients. Neopterin levels in the urine are elevated and those of BH_4 are decreased. This metabolic pattern may also be seen in PTS deficiency. The four patients reported all had elevated urinary excretion of primapterin (7-biopterin), which was the only persistent biochemical abnormality.

GENETICS AND PATHOGENESIS

Each of the defects in BH_4 metabolism is autosomal recessive. Consanguinity has been reported [39]. The overall frequency has been estimated at one in 106 births. In some groups, a higher incidence has been seen. In parts of Italy, these defects amount to 10 percent of all patients with hyperphenylalaninemia, in Turkey 15 percent, and in Taiwan 19 percent. In Saudi Arabia, the figure is 68 percent [35].

Levels of dihydropteridine reductase consistent with heterozygosity have been reported [51] in lymphocytes, lymphoblasts, and fibroblasts of parents. Obligate heterozygotes for GTP cyclohydrolase I deficiency were also found to have intermediate levels of activity [52]. In 6-PTS deficiency, heterozygotes tended to have quite low levels of enzyme activity and may be symptomatic [53]. Prenatal diagnosis has been carried out in DHPR deficiency by enzyme assay [54, 55]. Prenatal diagnosis has most commonly been carried out by the assessment of pterins in amniotic fluid [55, 56]. In GTPCH deficiency, levels of BH_4 and neopterin in amniotic fluid are very

low; in 6-PTS deficiency, BH_4 is low and neopterin high; in DHPR deficiency, BH_4 is high and neopterin normal or slightly elevated [55–58]. Affected and nonaffected fetuses have been diagnosed in this way in DHPR deficiency [57], in 6-PTS deficiency [55], and in GTPCH deficiency [57]. Molecular diagnosis by restriction fragment length polymorphism (RFLP) analysis of amniocytes has been reported in DHPR deficiency [59].

Four enzymatic defects have been described in this syndrome: DHPR deficiency [4], PCD deficiency [32], and the defects in the synthesis of BH_4 , GTPCH deficiency [39, 60], and 6-PTS deficiencies [38, 61, 62].

Each of these defects leads to a situation in which phenylalanine cannot be converted to tyrosine, even though the phenylalanine hydroxylase apoenzyme is normal. Tetrahydrobiopterin is also the cofactor for the hydroxylation of tryptophan and tyrosine. Thus, its deficiency interferes with the synthesis of serotonin, DOPA, and norepinephrine. Data have been obtained that indicate that this is the case, since levels of 5-hydroxyindoleacetic acid, vanillylmandelic acid, and homovanillic acid in the urine and cerebrospinal fluids are considerably lower than normal [33, 34]. Low levels of dopamine and serotonin have also been documented in the urine [6, 39]. Since it is possible in these disorders to have severe neurological disease in the presence of only mild hyperphenylalaninemia, levels of BH_4 may be relatively more sufficient for phenylalanine hydroxylation than that of tryptophan or tyrosine [33]. Defective neurotransmitter metabolism is doubtless related to the genesis of neurological abnormalities.

6-Pyruvoyltetrahydropterin synthase deficiency

Deficiency of 6-PTS is the most common of the defects in biopterin metabolism, approximating 60 percent of the patients. The majority have had the typical form, but there are a number in whom the presentation is atypical. The typical patients have high levels of neopterin and low biopterin in the urine (Table 16.1) and cerebrospinal fluid (CSF). These patients have the highest neopterin levels and the highest ratios of neopterin to BH_4 of all the abnormalities in pterin metabolism. The atypical patients have been referred to as peripheral because the CSF is normal, but with time it can become abnormal [63]. Clinical presentation in these patients is milder and

Table 16.1 Metabolite patterns in enzyme deficiencies

Defective enzyme	Urinary neopterin	Urinary biopterin
GTP cyclohydrolase	Absent	Absent
Pyruvoyltetrahydropterin synthetase	Elevated	Reduced
Dihydropteridine reductase	Normal	Elevated

response to treatment more satisfactory. A 20 mg/kg load of BH_4 leads to a rapid decrease in the plasma concentration of phenylalanine.

The enzyme 6-PTS was formerly called the 'phosphate eliminating enzyme', because it catalyzes the elimination of inorganic triphosphate from the dihydroneopterin triphosphate product of the cyclohydrolase reaction (Figure 16.3). This is an irreversible step. Markedly deficient activity was demonstrated first in biopsied liver [64]. The enzyme is expressed in erythrocytes and typical patients have less than 4 percent of control activity [65]. Patients with the atypical, peripheral form have partial activity in erythrocytes ranging from 5 to 23 percent of normal [66–68]. Residual activity does not always correlate with a milder phenotype; typical patients may have activities as high as 20 percent [53, 56]. Enzyme deficiency can also be documented in fibroblasts [69] in which activity is about 1 percent of control levels.

The reading frame of the cDNA for 6-PTS encompasses 435 bp over six exons. Twenty-eight mutations have been found distributed throughout all the exons of the gene [20, 70, 71]. Two were splice site mutations [69]. There were a few deletions and a majority were point mutations, some producing stop codons. N52S and P87S appear to be common in Asians. Mild hyperphenylalaninemia and dystonia were observed in a patient with a homozygous I114V mutation [72].

GTP cyclohydrolase deficiency

Defects in GTPCH account for about 4 percent of those with abnormalities in biopterin metabolism. In these patients, levels of both neopterin and biopterin are low, but their ratio may be normal [39, 52, 67, 73]. Concentrations of neurotransmitters and their metabolites 5-hydroxyindoleacetic acid and homovanillic acid are low. High concentrations of phenylalanine are corrected with BH_4 loading or replacement.

Defective enzyme activity has been documented in liver, lymphocytes [39, 68], and fibroblasts [74]. The human gene has been cloned and found to span 30 kb in six exons [18]. A mutation converting methionine to isoleucine at position 211 (M211 I) caused deficiency of the enzyme in a patient who was missed on neonatal screening [75]. Missense mutations, such as this and R184H, as well as nonsense mutations (Q110X) lead to complete deficiency of the enzyme. The gene (*GCHI*) is located at 14q22.1–22.2 [74].

Some 60 mutations have been reported. An abnormal gene on one allele may decrease enzyme activity to less than 50 percent, which may produce symptomatic patients and asymptomatic carriers.

In DOPA-dependent dystonia patients, the abnormality leads to partial deficiency of BH_4 , and subsequently of the activity of tyrosine hydroxylase (TH) [76]. Thus, partial deficiency leads to decreased dopamine. Early maturation of dopamine receptors may lead to age-dependent clinical manifestations.

Reduced activity of tyrosine hydroxylase in nigrostriatal dopamine neurons may lead to postural dystonia or postural tremor. Parkinson rigidity and resting tremor do not occur. The neuropathology of this disease is striking for an absence of degenerative changes.

Dihydropteridine reductase deficiency

DHPR deficiency accounts for approximately a third of the patients with defects in biopterin metabolism. Levels of neurotransmitters are low in CSF and urine (Table 16.1). These include homovanillic acid (HVA), vanillylmandelic acid (VMA), 3-methoxyhydroxyphenylglycol (MHPG), and 5-hydroxyindoleacetic acid (HIAA); the metabolites of dopamine, norepinephrine, epinephrine, and serotonin, respectively [77]. Total urinary pterins are elevated and BH_4 is low.

Deficient activity of the enzyme has been documented in liver, brain, and cultured fibroblasts [4]. The enzyme can also be assayed in erythrocytes [78]. Activity is generally very low, and some patients are cross-reacting material (CRM)-positive and some CRM-negative without correlation with degrees of clinical severity [79–81].

The gene was mapped to chromosome 4p15.31 [82]. The intron/exon structure has been determined for the gene that codes for a 25.7-kDa protein [83, 84]. A number of mutations have been identified spread throughout *QDPR* (the gene). An insertion of an extra codon for threonine between alanine at position 122 and the threonine at residue 123 [22] was an early identification, but accounts for most of the mutations reported [85]. A number of RFLPs has been identified in the gene, which may be useful for prenatal diagnosis and population genetic studies [59, 83]. More than 25 different mutations have been reported. Mutational analysis is the best method for prenatal diagnosis in a family with or a known mutation [86].

Pterin-4 α -carbinolamine dehydratase deficiency

PCD deficiency occurs in about 4 percent of patients with defective BH_4 metabolism [27]. When the phenylalanine hydroxylation reaction takes place, the carbinolamine intermediate is converted to dihydropterin in a reaction catalyzed by the dehydratase PCD. When PCD is defective, there is a conversion to 7-biopterin (primapterin), and the excretion of this compound is a distinguishing characteristic of this disorder [87]. The dehydratase reaction can also proceed nonenzymatically [88] and this could be a reason for the relatively mild phenotype.

PCD is a bifunctional protein with transcriptional function; its gene codes for four exons over 5 kb [89]. Many mutations described have been nonsense mutations, but they have clustered in exon 4 [71].

DIAGNOSIS

Although only 2 percent of the patients found to have hyperphenylalaninemia have disorders of bipterin metabolism, every patient should be tested for an abnormality of BH₄ because the implications for management and counseling are so different [89].

The diagnosis of these disorders may be made in a number of ways. Among the simplest is the administration of tetrahydrobiopterin [44]. Doses of 2 mg/kg intravenously and 7.5–20 mg/kg orally have been recommended [46]. Administration of BH₄ leads to a prompt decrease to normal in the concentration of phenylalanine in patients with synthesis and reductase defects, and of course no change in the patient with PKU. It is important that the patient be on a diet containing normal amounts of phenylalanine, not the therapeutic diet employed for patients with PKU. A few patients with DHPR defects have been missed using the BH₄ loading test [90, 91]. Loading tests must be confirmed by analysis of enzyme activity in any abnormality of BH₄ metabolism. The gold standard for the diagnosis of DHPR deficiency is assay of the enzyme.

Currently, routine testing for patients with hyperphenylalaninemia includes the assay of DHPR activity in dried spots on Guthrie cards [51, 92, 93]. Definitive testing for activity can be accomplished in cultured fibroblasts, lymphoblasts, or freshly isolated lymphocytes. The defect has also been demonstrated in biopsied liver [4, 33]. The other test that has become routine for the detection of BH₄ abnormalities in hyperphenylalaninemic infants is the assay of pterin metabolites in the urine.

Pterin metabolites in urine are measured by high performance liquid chromatography (HPLC) [94, 95]. The normal values for bipterin and neopterin are 0.4–2.5 and 0.1–5.0 mmol/mol creatinine, respectively, and the proportion of bipterin is 20–80 percent [96]. In those with defective GTPCH, all pterins are low in blood and urine and the ratio is normal [39, 60]. In patients with 6PTS deficiency, the concentrations of bipterin are very low and those of neopterin high [46]. In PCD deficiency, primapterin is formed in the urine, and BH₄ is low. In patients with DHPR deficiency, there is a lack of feedback inhibition, and so there may be massive overproduction of urinary pterins, but the level of BH₄ is always low. On the other hand, patients with DHPR deficiency have been reported in whom urinary pterin analysis was normal [97], indicating further the importance of enzyme analysis in the diagnosis of this condition. The normal plasma BH₄ value is 1.4–3.0; that of blood 2.4–6.0 ng/mL [96].

TREATMENT

Patients with defects in pterin metabolism, especially those with abnormalities in BH₄ synthesis should be treated with BH₄ (Figure 16.14) [6, 44, 96–98]. This compound is available from B Schircks (Ch 8641, Jona, Switzerland) and

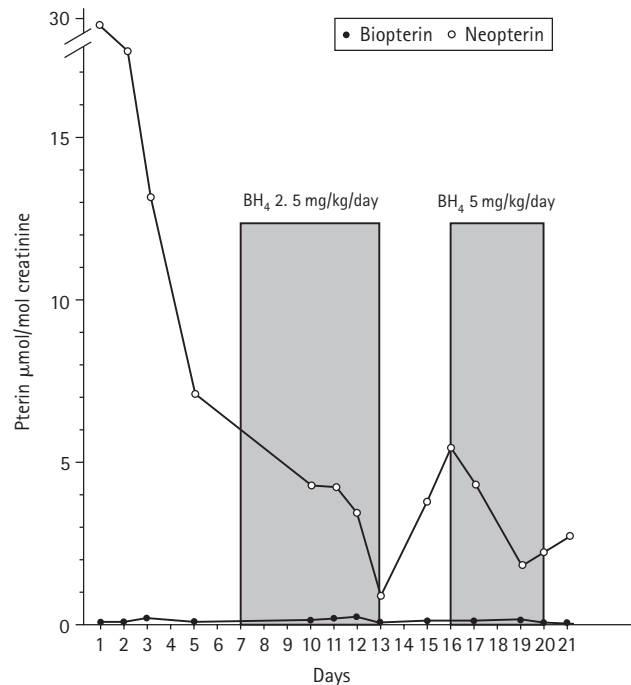


Figure 16.14 Treatment of 6PTS deficiency with BH₄ reduced the excretion of neopterin in the urine.

from Suntory (Tokyo, Japan). It is available in the United States as kuvan (Biomarin). It is given daily in large doses (2–20 mg/kg) [46, 99, 100]. BH₄ tablets contain 100-mg BH₄. Protein intake is also restricted. BH₄ treatment may reduce concentrations of tyrosine in plasma and CSF. Levels may be monitored and supplemental tyrosine (as in PKU formulations) employed if necessary.

In BH₄ synthesis defects, a single daily dose of 2–5 mg/kg is usually sufficient to control levels of phenylalanine. In DHPR defects, larger doses (20 mg/kg) may be required, and it must be given fractionally through the day. Some patients with DHPR deficiency must be treated with sufficient restriction of phenylalanine intake to maintain normal plasma levels [90, 101].

Sapropterin dihydrochloride (kuvan) is the synthetic analog of BH₄. It is the 6R-isomer. It is safe and well tolerated. Adverse event rates were similar to those of placebo [102]. It is Food and Drug Administration (FDA) approved for disorders of biotin synthesis, as well as for PKU.

In addition to controlling the concentration of

Table 16.2 BH₄ is the cofactor for three enzymatic reactions

L-Phenylalanine + BH ₄	→	L-Tyrosine + BH ₂
L-Tyrosine + BH ₄	→	L-Dopa + BH ₂
L-Tryptophan + BH ₄ -L-5	→	Hydroxytryptophan + BH ₂

phenylalanine, treatment of this condition must correct deficiencies of neurotransmitters (Table 16.2). This cannot be done with BH₄ alone, although it is clear that it does enter the CSF [103, 104].

Neurotransmitter balance is treated with a regimen of biogenic amine precursors including 5-hydroxytryptophan and L-DOPA along with carbidopa [105, 106], which inhibits peripheral decarboxylation permitting entry to the central nervous system (CNS), where decarboxylation to serotonin and dopamine takes place. Preparations, such as Sinemet, which combine L-DOPA and carbidopa may be useful. Treatment with L-DOPA, carbidopa, and 5-hydroxytryptophan (serotonin) is introduced slowly and sequentially; steps are 1 mg/kg over days or weeks. L-DOPA is given in doses of 8–12 mg/kg and 5-hydroxytryptophan 6–9 mg/kg. Carbidopa is 10–20 percent of DOPA in most formulations. In some patients, a fixed preparation of carbidopa may have to be altered to give more or less carbidopa.

Measurements of neurotransmitter metabolites and pterins in the cerebrospinal fluid are required in order to determine optimal doses and to monitor the effectiveness of therapeutic regimens. The level of prolactin in serum is elevated in these diseases, which is an index of abnormal dopamine homeostasis, and it may be useful to monitor levels in order to guide therapy [101]. Levels of folate may be low in DHPR deficiency, and DHPR appears to have a role in tetrahydrofolate synthesis [107, 108]. Folinic acid has been employed in a dose of 12.5 mg/day [107].

Progressive improvement, with disappearance of myoclonus, involuntary movements, and tetraplegia, has been reported following treatment with biogenic amine precursors without BH₄ [4, 30], but this would not be recommended. There is evidence that early treatment may prevent progression [35, 46], but overall experience indicates that prognosis should be guarded. Many patients have considerable neurological impairment despite therapy. Programs must be individually tailored to meet the needs of the patient.

Effects of treatment were assessed in a group of Japanese patients with BH₄ deficiency to whom a comprehensive battery of neuropsychological tests were administered [109]. This uncovered deficits in executive function, which has been thought to be controlled by prefrontal dopaminergic systems. Patients treated from birth to 2.5 years had normal executive functioning, while five treated later performed poorly.

In an Italian study of (6-PTS) deficiency [110], patients were divided into those with normal or markedly depressed levels of biogenic amines in the CSF. All patients with the mild form were neurologically normal, except for one with transient dystonia, while all with the severe form had impressive neurological impairment.

In another study, a dopamine agonist (pramipexole) was used twice daily as an adjuvant of DOPA therapy [111]. The regimen also included selegiline, an irreversible monoamine oxidase (MAO-B) inhibitor, and entacapone,

a catechol-O-methyl transferase inhibitor. They reported better results than L-DOPA treatment alone and appreciable reduction in the amounts of DOPA employed.

Treatment of DHPR deficiency is clearly demanding. A good result is by no means guaranteed even if treatment follows all of the guidelines. The Swiss experience indicates that DHPR deficiency is more of a problem than PTPS deficiency. The onset of treatment appears to determine outcome [112].

Maternal PTPS deficiency was documented in a woman who had two pregnancies [113]. In each, doses of neurotransmitter precursors and BH₄ were increased. The first led to a girl who was said to have above average intellectual development despite a left hemiparesis and absence of the corpus callosum and right-sided schizencephaly. The second, a boy, had no anatomic abnormalities and was described as normal at five years of age.

Treatment of DOPA-responsive dystonia with levodopa is enormously rewarding and can lead to a complete disappearance of the motor abnormalities [76]. If begun early enough, it can reverse the shortness of stature.

REFERENCES

1. Kaufman S. A new cofactor required for the enzymatic conversion of phenylalanine to tyrosine. *J Biol Chem* 1958; **230**: 931.
2. Bartholome K. A new molecular defect in phenylketonuria. *Lancet* 1974; **2**: 1580.
3. Smith I. Atypical phenylketonuria accompanied by a severe progressive neurological illness unresponsive to dietary treatment. *Arch Dis Child* 1974; **49**: 245.
4. Kaufman S, Holtzman NA, Milstein S et al. Phenylketonuria due to a deficiency of dihydropteridine reductase. *N Engl J Med* 1975; **293**: 785.
5. Longo N. Disorders of biopterin metabolism. *J Inherit Metab Dis* 2009; **32**: 333.
6. Al Aqeel A, Gascon G, Ozand PT. Malignant hyperphenylalaninemia. CT and MRI of the brain. *Am J Neuroradiol* 1990; **90**: 135.
7. Kaufman S. Metabolism of phenylalanine hydroxylation cofactor. *J Biol Chem* 1967; **242**: 3943.
8. Lei XD, Kaufman S. Human white blood cells and hair follicles are good sources of mRNA for the pterin carbinolamine dehydratase/dimerization cofactor of HNF for mutation detection. *Biochem Biophys Res Commun* 1998; **248**: 432.
9. Craine JE, Hall ES, Kaufman S. The isolation and characterization of dihydropteridine reductase from sheep liver. *J Biol Chem* 1972; **247**: 6082.
10. Brown GM. The biosynthesis of pteridines. *Adv Enzymol* 1971; **35**: 35.
11. Eto I, Fukushima K, Shioa T. Enzymatic synthesis of biopterin from D-erythro-dihydroneopterin triphosphate by extracts of kidneys from Syrian golden hamsters. *J Biol Chem* 1976; **251**: 6505.

12. Gal EM, Nelson JM, Sherman AD. Biopterin: III. Purification and characterization of enzymes involved in the cerebral synthesis of 78-dihydrobiopterin. *Neurochem Res* 1978; **3**: 69.
13. Burg AW, Brown GM. The biosynthesis of folic acid: VIII. Purification and properties of the enzyme that catalyzes the production of formate from carbon 8 of guanosine triphosphate. *J Biol Chem* 1968; **243**: 2349.
14. Takikawa S, Curtius H-C, Redweik U, Ghisla S. Purification of 6-pyruvoyl tetra-hydropterin synthase from human liver. *Biochem Biophys Res Commun* 1986; **134**: 646.
15. Takikawa S-I, Curtius H-C, Redweik U *et al*. Biosynthesis of tetrahydrobiopterin. Purification and characterization of 6-pyruvoyl-tetrahydropterin synthase from human liver. *Eur J Biochem* 1986; **161**: 295.
16. Dahl HHM, Hutchinson W, McAdam W *et al*. Human dihydropteridine reductase: characterization of a cDNA clone and its use in analysis of patients with dihydropteridine reductase deficiency. *Nucleic Acids Res* 1987; **15**: 1921.
17. Lockyer J, Cook RG, Milstein S *et al*. Structure and expression of human dihydropteridine reductase. *Proc Natl Acad Sci USA* 1987; **84**: 3329.
18. Nomura T, Ichinose H, Sumi-Ichinose C *et al*. Cloning and sequencing of cDNA encoding mouse GTP cyclohydrolase I. *Biochem Biophys Res Commun* 1993; **191**: 523.
19. Hatakayama K, Ashida A, Owada M *et al*. Molecular basis of malignant hyperphenylalaninemia: a mutation in the gene encoding human 6-pyruvoyl-tetra-hydropterin synthase. First International Union of Biochemistry and Molecular Biology, 1992: 2-a-05-P7 (Abstr.).
20. Oppliger T, Thony B, Leimbacher W *et al*. Structural and functional consequences of mutations in 6-pyruvoyltetra-hydropterin synthase causing hyperphenyl-alaninemia in man. SSIEM Symposium 1995; **37**: P026.
21. Kluge C, Brecevic L, Heizmann CW *et al*. Chromosomal localization genomic structure and characterization of a single human gene (PTS) and retropseudogene for 6-pyruvoyltetrahydropterin synthase. *Eur J Biochem* 1996; **240**: 477.
22. Thony B, Heizmann CW, Mattei MG. Chromosomal location of two human genes encoding tetrahydrobiopterin-metabolizing enzymes: 6-pyruvoyl-tetrahydropterin synthase maps to 11q223-q233 and pterin-4a-carbinolamine dehydratase maps to 10q22. *Genomics* 1994; **19**: 365.
23. Howells DW, Forrest SM, Dahl HHM, Cotton RGH. Insertion of an extra codon for threonine is a cause of dihydropteridine reductase deficiency. *Am J Hum Genet* 1990; **47**: 279.
24. Citron BA, Kaufman S, Milstien S *et al*. Mutation in the 4a-carbinolamine dehydratase gene leads to mild hyperphenylalaninemia with defective cofactor metabolism. *Am J Hum Genet* 1993; **53**: 768.
25. Ichinose H, Ohye T, Matsuda Y *et al*. Characterization of mouse and human GTP cyclohydrolase I genes: mutations in patients with GTP cyclohydrolase I deficiency. *J Biol Chem* 1995; **270**: 10062.
26. Thöny B, Blau N. Mutations in the GTP cyclohydrolase I and 6-pyruvoyl-tetrahydropterin synthase genes. *Hum Mutat* 1997; **10**: 11.
27. Blau N, Barnes I, Dhondt JL. International database of tetrahydrobiopterin deficiencies. *J Inherit Metab Dis* 1995; **19**: 8.
28. Ichinose H, Inagaki H, Suzuki T *et al*. Molecular mechanisms of hereditary progressive dystonia with marked diurnal fluctuation Segawa's disease. *Brain Dev* 2000; **22**(Suppl. 1): S107.
29. Thony B, Blau N. Mutations in the BH4-metabolizing genes GTP cyclohydrolase I, 6-pyruvoyl-tetrahydropterin synthase, sepiapterin reductase, carbinolamine-4a-dehydratase, and dihydropteridine reductase. *Hum Mutat* 2006; **27**: 870.
30. Dhondt J, Guibaud P, Rolland M *et al*. Neonatal hyperphenylalaninemia presumably caused by a new variant of biopterin synthetase deficiency. *Eur J Pediatr* 1988; **147**: 153.
31. Blaskovics M, Guidici T. A new variant of biopterin deficiency. *N Engl J Med* 1988; **319**: 1611.
32. Blau N, Curtius H-C, Kuster T *et al*. Primapterinuria: a new variant of atypical phenylketonuria. *J Inherit Metab Dis* 1989; **12**(Suppl. 2): 335.
33. Brewster TG, Moskowitz HA, Kaufman S *et al*. Dihydropteridine reductase deficiency associated with severe neurologic disease and mild hyperphenylalaninemia. *Pediatrics* 1979; **63**: 94.
34. Smith I, Clayton BE, Wolff OH. New variant of phenylketonuria with progressive neurological illness unresponsive to phenylalanine restriction. *Lancet* 1975; **1**: 328.
35. Al Aqeel A, Ozand PT, Gascon G *et al*. Biopterin-dependent hyperphenylalaninemia due to deficiency of 6-pyruvoyl tetrahydropterin synthase. *Neurology* 1991; **41**: 730.
36. Narisawa K, Arai N, Ishizawa S *et al*. Dihydropteridine reductase deficiency: diagnosis by leukocyte enzyme assay. *Clin Chim Acta* 1980; **105**: 3351.
37. Endres W, Niederwieser A, Curtius H-Ch *et al*. Atypical phenylketonuria due to biopterin deficiency. *Helv Paediatr Acta* 1982; **37**: 489.
38. Allen RJ, Young W, Bonacci J *et al*. Neonatal dystonic parkinsonism a 'stiff baby syndrome' in biopterin deficiency with hyperprolactinemia detected by newborn screening for hyperphenylalaninemia and responsiveness to treatment. *Ann Neurol* 1990; **28**: 434.
39. Niederwieser A, Blau N, Wang M *et al*. GTP cyclohydrolase I deficiency a new enzyme defect causing hyperphenylalaninemia with neopterin biopterin dopamine and serotonin deficiencies and muscular hypotonia. *Eur J Pediatr* 1984; **141**: 208.
40. Butler IJ, O'Flynn ME, Seifert WE, Howell RR. Neurotransmitter defects and treatment of disorders of hyperphenylalaninemia. *J Pediatr* 1981; **98**: 729.
41. Brismar J, Al Aqeel A, Gascon G, Ozand P. Malignant hyperphenylalaninemia: CT and MR of the brain. *Am J Neuroradiol* 1990; **11**: 135.
42. Smith I, Leeming RJ, Cavanagh NP, Hyland K. Neurological aspects of biopterin metabolism. *Arch Dis Child* 1986; **61**: 130.
43. Gudinchet F, Maeder P, Meuli RA *et al*. Cranial CT and MRI in malignant phenylketonuria. *Pediatr Radiol* 1992; **22**: 223.
44. Danks DM, Schlesinger P, Firgaira F *et al*. Malignant hyperphenylalaninemia – clinical features biochemical findings and experience with administration of biopterins. *Pediatr Res* 1979; **13**: 1150.

45. Matalon R. Screening for bipterin defects: experience with 387 patients with hyperphenylalaninemia. Twenty-second meeting of SSIEM, Newcastle, England, September 4–7, 1984.
46. Al Aqeel A, Ozand PT, Gascon GG *et al.* Response of 6-pyruvoyl-tetrahydropterin synthase deficiency (6PTSD) to tetrahydrobiopterin. *J Child Neurol* 1992; **7**: S26.
47. Al Aqeel A, Ozand PT, Gascon GG. Hyperphenylalaninemia. *Neurology* 1992; **40**: 704.
48. Bandmann O, Wood NW. Dopa-responsive dystonia. *Neuropediatrics* 2002; **33**: 1.
49. Blau N, Bonafe L, Thony B. Tetrahydrobiopterin deficiencies without hyperphenylalaninemia: diagnosis and genetics of dopa-responsive dystonia and sepiapterin reductase deficiency. *Mol Genet Metab* 2001; **74**: 172.
50. Thony B, Neuheiser F, Kierat L *et al.* Mutations in the pterin-4 α -carbinolamine dehydratase (PCBD) gene cause a benign form of hyperphenylalaninemia. *Hum Genet* 1998; **103**: 162.
51. Firgaira FA, Cotton RGH, Danks DM. Dihydropteridine reductase deficiency diagnosis by assays on peripheral blood cells. *Lancet* 1979; **2**: 1260.
52. Naylor EW, Ennis D, Davidson AGF *et al.* Guanosine triphosphate cyclohydrolase I deficiency: early diagnosis by routine urine pteridine screening. *Pediatrics* 1987; **79**: 374.
53. Scriver CR, Clow CL, Kaplan P, Niederwieser A. Hyperphenylalaninemia due to deficiency of 6-pyruvoyl tetrahydropterin synthase. Unusual gene dosage effect in heterozygotes. *Hum Genet* 1987; **77**: 168.
54. Guardamagna O, Spada M, Ponzone A *et al.* Prenatal diagnosis of dihydropteridine reductase deficiency in a twin pregnancy. *Pteridines* 1992; **3**: 19.
55. Blau N, Niederwieser A, Curtius H-C *et al.* Prenatal diagnosis of atypical phenylketonuria. *J Inherit Metab Dis* 1989; **12**: 295.
56. Blau N, Kierat L, Matasovic A *et al.* Antenatal diagnosis of tetrahydrobiopterin deficiency by quantification of pterins in amniotic fluid and enzyme activity in fetal and extrafetal tissue. *Clin Chim Acta* 1994; **226**: 159.
57. Dhondt JL, Tilmont P, Ringel J *et al.* Pterins [AU108] analysis in amniotic fluid for the prenatal diagnosis of GTP cyclohydrolase deficiency. *J Inherit Metab Dis* 1990; **13**: 879.
58. Niederwieser A, Shintaku H, Hasler T *et al.* Prenatal diagnosis of 'dihydrobiopterin synthetase' deficiency a variant form of phenylketonuria. *Eur J Pediatr* 1986; **145**: 176.
59. Dahl HHM, Wake S, Cotton RGH, Danks DM. The use of restriction fragment length polymorphism in prenatal diagnosis of dihydropteridine reductase deficiency. *J Med Genet* 1988; **25**: 25.
60. Dhondt JL, Farriaux JP, Boudha A *et al.* Neonatal hyperphenylalaninemia presumably caused by guanosine triphosphate cyclohydrolase deficiency. *J Pediatr* 1985; **106**: 954.
61. Niederwieser A, Curtius H-Ch, Bettoni O *et al.* Atypical phenylketonuria caused by 78-dihydrobiopterin synthetase deficiency. *Lancet* 1979; **1**: 131.
62. Dhondt JL. Strategy for the screening of tetrahydrobiopterin deficiency among hyperphenylalaninemic patients: 15-years experience. *J Inherit Metab Dis* 1991; **14**: 117.
63. Ponzone A, Blau N, Guardamagna O *et al.* Progression of 6-pyruvoyl-tetrahydropterin synthase deficiency from a peripheral into a central phenotype. *J Inherit Metab Dis* 1990; **13**: 298.
64. Niederwieser A, Leimbacher W, Curtius H-C *et al.* Atypical phenylketonuria with dihydrobiopterin synthetase deficiency: absence of phosphate-eliminating enzyme activity demonstrated in liver. *Eur J Pediatr* 1985; **144**: 13.
65. Niederwieser A, Shintaku H, Hasler TH *et al.* Prenatal diagnosis of 'dihydropterin synthetase' deficiency a variant form of phenylketonuria. *Eur J Pediatr* 1986; **145**: 176.
66. Niederwieser A, Shintaku H, Leimbacher W *et al.* Peripheral tetrahydrobiopterin deficiency with hyperphenyl-alaninemia due to incomplete 6-pyruvoyl tetrahydropterin synthase deficiency or heterozygosity. *Eur J Pediatr* 1987; **146**: 228.
67. Dhondt JL, Farriaux JP, Boudha A *et al.* Neonatal hyperphenylalaninemia presumably caused by guanosine triphosphate cyclohydrolase deficiency. *J Pediatr* 1985; **106**: 954.
68. Blau N, Niederwieser A. Guanosine triphosphate cyclohydrolase I assay in human and rat liver using high-performance liquid chromatography of neopterin phosphates and guanine nucleotides. *Anal Biochem* 1983; **128**: 446.
69. Oppliger T, Thony B, Kluge C *et al.* Identification of mutations causing 6-pyruvoyl-tetrahydropterin synthase deficiency in four Italian families. *Hum Mutat* 1997; **10**: 25.
70. Romstad A, Guldberg P, Levy HL *et al.* Single-step mutation scanning of the 6-pyruvoyl-tetrahydropterin synthase gene in patients with hyperphenylalaninemia. *Clin Chem* 1999; **45**: 2102.
71. Blau N, Thony B, Diansani I. BIOMDB: Database of mutations causing tetrahydrobiopterin deficiency. Last accessed October 2004. Available from: www.bh4.org.
72. Hanihara T, Inoue K, Kawanishi C *et al.* 6-Pyruvoyl-tetrahydropterin synthase deficiency with generalized dystonia and diurnal fluctuation of symptoms: a clinical and molecular study. *Mov Disord* 1997; **12**: 408.
73. Coskun T, Karagoz T, Kalkanoglu S *et al.* Guanosine triphosphate cyclohydrolase I deficiency. A rare cause of hyperphenylalaninemia. *Tur J Pediatr* 1999; **41**: 231.
74. Milstein S, Kaufman S, Sakai N. Tetrahydrobiopterin biosynthesis defects examined in cytokine-stimulated fibroblasts. *J Inherit Metab Dis* 1993; **16**: 975.
75. Blau N, Ichinose H, Nagatsu T *et al.* A missense mutation in a patient with guanosine triphosphate cyclo-hydrolase deficiency missed in the newborn screening program. *J Pediatr* 1995; **126**: 401.
76. Segawa M. Hereditary progressive dystonia with marked diurnal fluctuation. *Brain Dev* 2000; **22**(Suppl. 1): S65.
77. Niederwieser A, Ponzone A, Curtius H-C. Differential diagnosis of tetrahydrobiopterin deficiency. *J Inherit Metab Dis* 1985; **8**: 34.
78. Narisawa K, Arai N, Hayakawa H, Tada K. Diagnosis of dihydropteridine reductase deficiency by erythrocyte enzyme assay. *Pediatrics* 1981; **68**: 591.
79. Cotton RGH, Jennings I, Bracco G *et al.* Tetrahydrobiopterin non-responsiveness in dihydropteridine reductase deficiency is associated with the presence of mutant protein. *J Inherit Metab Dis* 1986; **9**: 239.

80. Ponzzone A, Guardamagna O, Bracco G *et al.* Two mutations of dihydropteridine reductase deficiency. *Arch Dis Child* 1988; **63**: 154.
81. Firgaira FA, Choo KH, Cotton RGH, Danks DM. Molecular and immunological comparison of human dihydropteridine reductase in liver cultured fibroblasts and continuous lymphoid cells. *Biochem J* 1981; **197**: 45.
82. Sumi S, Ishikawa T, Ito Y *et al.* Probable assignment of the dihydropteridine reductase gene to 4p1531. *Tohoku J Exp Med* 1990; **160**: 93.
83. Smooker PM, Howells DW, Cotton RGH. Dihydropteridine reductase deficiency-D – identification of natural mutations and analysis by recombinant expression and *in vivo* protein studies. *Am J Hum Genet* 1991; **49**(Suppl.): A193
84. Dianzani I, De Santis L, Smooker PM *et al.* Dihydropteridine reductase deficiency: physical structure of the QDPR gene identification of two new mutations and genotype-phenotype correlations. *Hum Mutat* 1998; **12**: 267.
85. De Sanctis L, Alliaudi C, Spada M *et al.* Genotype-phenotype correlation in dehydropteridine reductase deficiency. *J Inher Metab Dis* 2000; **23**: 333.
86. Kalkanoglu HS, Romstad A, Coskun T *et al.* Evaluation of a fetus at risk for dihydropteridine reductase deficiency by direct mutation analysis using denaturing gradient gel electrophoresis. *Prenat Diagn* 2001; **21**: 868.
87. Curtius H-Ch, Kuster T, Matasovic A *et al.* Primapterin anapterin and 6-oxo-primapterin three new 7-substituted pterins identified in a patient with hyperphenyl-alaninemia. *Biochem Biophys Res Commun* 1988; **153**: 715.
88. Curtius H-Ch, Adler C, Rebrin I *et al.* 7-Substituted pterins; formation during phenylalanine hydroxylation in the absence of dehydratase. *Biochem Biophys Res Commun* 1990; **172**: 1060.
89. Thony B, Neuheiser F, Blau N, Heizmann CW. Characterization of the human PCBD gene encoding the bifunctional protein pterin-4a-carbinolamine dehydratase/dimerization cofactor for the transcription factor HNF-1 alpha. *Biochem Biophys Res Commun* 1995; **210**: 966.
90. Lipson A, Yu J, O'Halloran M *et al.* Dihydropteridine reductase deficiency: non-response to oral tetrahydrobiopterin load test. *J Inher Metab Dis* 1984; **7**: 69.
91. Endres W, Ibel H, Kierat L *et al.* Tetrahydrobiopterin and 'non-responsive' dehydropteridine reductase deficiency. *Lancet* 1987; **2**: 223.
92. Sahota A, Blair JA, Barford PA *et al.* Neonatal screening for dihydropteridine reductase deficiency. *J Inher Metab Dis* 1985; **8**: 99.
93. Arai N, Narisawa K, Hayakawa H, Taka K. Hyperphenylalaninemia due to dehydropteridine reductase deficiency: diagnosis by enzyme assay on dried blood spots. *Pediatrics* 1982; **98**: 426.
94. Niederwieser A, Curtius HC, Gitzelmann R *et al.* Excretion of pterins in phenylketonuria and phenylketonuria variants. *Helv Paediatr Acta* 1980; **35**: 335.
95. Howells DW, Smith I, Hyland K. Estimation of tetrahydrobiopterin and other pterins in cerebrospinal fluid using reversed-phase high-performance liquid chromatography with electrochemical and fluorescence detection. *J Chromatogr* 1986; **381**: 285.
96. Smith I. Disorders of tetrahydrobiopterin metabolism. In: Fernandes J, Saudubray J-M, Tada K (eds). *Inborn Metabolism Diseases Diagnosis and Treatment*. Berlin: Springer-Verlag, 1991: 183.
97. Blau N, Heizmann CW, Sperl W *et al.* Atypical (mild) forms of dihydropteridine reductase deficiency: neurochemical evaluation and mutation detection. *Pediatr Res* 1992; **32**: 726.
98. Curtius H-C, Niederwieser A, Visconti M *et al.* Atypical phenylketonuria due to tetrahydrobiopterin deficiency. Diagnosis and treatment with tetrahydrobiopterin dihydrobiopterin and sepiapterin. *Clin Chim Acta* 1979; **93**: 251.
99. Ponzzone A, Guardamagna O, Dianzani I *et al.* Catalytic activity of tetrahydrobiopterin in dihydropteridine reductase deficiency and indications for treatment. *Pediatr Res* 1993; **33**: 125.
100. Smith I, Hyland K, Kendall B, Leeming R. Clinical role of pteridine therapy in tetrahydrobiopterin deficiency. *J Inher Metab Dis* 1985; **8**: 39.
101. Spada M, Ferraris S, Altare F *et al.* Monitoring treatment in tetrahydrobiopterin deficiency by serum prolactin. SSIEM Symposium 1995; **33**: P029.
102. Hegge KA, Horning KK, Peitz GJ *et al.* Sapropterin: a new therapeutic agent for phenylketonuria. *Ann Pharmacol* 2009; **43**: 1466.
103. Kapatos G, Kaufman S. Peripherally administered reduced pterins do enter the brain. *Science* 1981; **212**: 955.
104. Hoshiga M, Hatakeyama K, Watanabe M *et al.* Autoradiographic distribution of [¹⁴C]tetrahydrobiopterin and its developmental change in mice. *J Pharmacol Exp Ther* 1993; **267**: 971.
105. Bartholome K, Byrd DJ. L-DOPA and 5-hydroxytryptophan therapy in phenylketonuria with normal phenylalanine hydroxylase. *Lancet* 1975; **2**: 1042.
106. Endres W. Biopterin deficiency. Therapy of tetrahydrobiopterin deficiencies monotherapy of combined treatment with neurotransmitter precursors. In: Bickel H, Wachtel U (eds). *International Symposium on Recent Progress in the Understanding Recognition and Management of Inherited Diseases of Amino Acid Metabolism*. Stuttgart: Georg Thieme, 1985: 124.
107. Irons M, Levy HL, O'Flynn E *et al.* Folinic acid therapy in treatment of dihydropteridine reductase deficiency. *J Pediatr* 1987; **110**: 61.
108. Pollock RJ, Kaufman S. Dihydropterine reductase may function in tetrahydrofolate metabolism. *J Neurochem* 1978; **31**: 115.
109. Tanaka Y, Kato M, Muramatsu T. Early initiation of L-dopa therapy enables stable development of executive function in tetrahydrobiopterin BH4 deficiency. *Dev Med Child Neurol* 2007; **49**: 372.
110. Leuzzi V, Carducci CA, Carducci CL *et al.* Phenotypic variability, neurological outcome and genetics background of 6-pyruvoyl-tetrahydropterin synthase deficiency. *Clin Genet* 2010; **3**: 249.
111. Porta F, Mussa A, Concoline D *et al.* Dopamine agonists in 6-pyruvoyl tetrahydropterin synthase deficiency. *Neurology* 2009; **73**: 633.

112. Jaggi L, Zurfluh MR, Schuler A *et al.* Outcome and long-term follow-up of 36 patients with tetrahydrobiopterin deficiency. *Mol Genet Metab* 2008; **93**: 295.
113. Gizewska M, Hnatyszyn G, Sagan L *et al.* Maternal tetrahydrobiopterin deficiency: the course of two pregnancies and follow-up of two children in a mother with 6-pyruvoyl-tetrahydropterin synthase deficiency. *J Inherit Metab Dis* 2009; Mar 30 [Epub ahead of print].

Biogenic amines

Introduction	136	Treatment	140
Clinical abnormalities	136	Introduction	141
Genetic and pathogenesis	139	Clinical abnormalities	141
Treatment	139	Genetics and pathogenesis	141
Introduction	139	Treatment	142
Clinical picture	140	References	142
Genetics and pathogens	140		

The disorders of BH₄ synthesis ([Chapter 25](#)) lead to abnormalities with the biogenic amine neurotransmitters. Other disorders that lead to neurotransmitter imbalance are aromatic L-amino acid decarboxylase (AADC) deficiency, which also leads to both catecholamine and serotonin deficiency, and tyrosine hydroxylase (TH) deficiency, which causes catecholamine deficiency. These disorders are included in this chapter, which also includes dihydrofolate reductase (DHPR) deficiency, another cause of defective synthesis of BH₄.

Aromatic L-amino acid decarboxylase deficiency

MAJOR PHENOTYPIC EXPRESSION

Hypotonia, oculogyric crises, developmental impairment, dystonia, excessive sweating or temperature instability, reduced concentrations in the cerebrospinal fluid (CSF) of 5-hydroxyindoleacetic acid (HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylglycol (MHPG) along with elevated concentrations of 5-hydroxytryptophan and 3-O-methyldopa; and decreased activity of AADC.

INTRODUCTION

AADC deficiency was first reported by Hyland and Clayton [1] and Hyland *et al.* [2] in twins who were very hypotonic, and had interspersed bouts of crying and paroxysmal movements of the arms and legs along with oculogyric crises. They developed abnormalities in temperature regulation and postural hypotension. Concentrations of HIAA and HVA in the CSF were very low. Serotonin in whole blood and catecholamines in plasma were also low. Elevated amounts of L-DOPA, 5-hydroxytryptophan (HTP) and 3-methoxytyrosine were found in the urine. The activity of AADC ([Figure 17.1](#)) was close to zero (1 percent of control) in plasma and liver.

Serotonin and dopamine are formed after the hydroxylation of tryptophan and tyrosine catalyzed by tryptophan hydroxylase and TH ([Figure 17.1](#)) followed by decarboxylation of HTP and L-DOPA by pyridoxalphosphate (PLP)-dependent AADC.

The disorder is relatively rare, but 78 patients have been tabulated in a database of pediatric neurotransmitter disorders [3].

In six patients with deficiency of AADC, six point mutations were identified [4], four homozygous and two compound heterozygous. Homozygosity for a mutation in the AADC was found in both the original twins of Hyland and Clayton [5]. A total of 24 mutations has been found in the 49 patients [3]. The most common mutation, found in 45 percent of alleles, was IVS6+4A>T [3].

CLINICAL ABNORMALITIES

Onset of clinical manifestations is virtually always (96 percent) [3] in infancy or childhood. Most (95 percent) present with hypotonia. This may alternate in some with hypertonia. Oculogyric crises represent a common (86 percent) and memorable feature of the disease ([Figures](#)

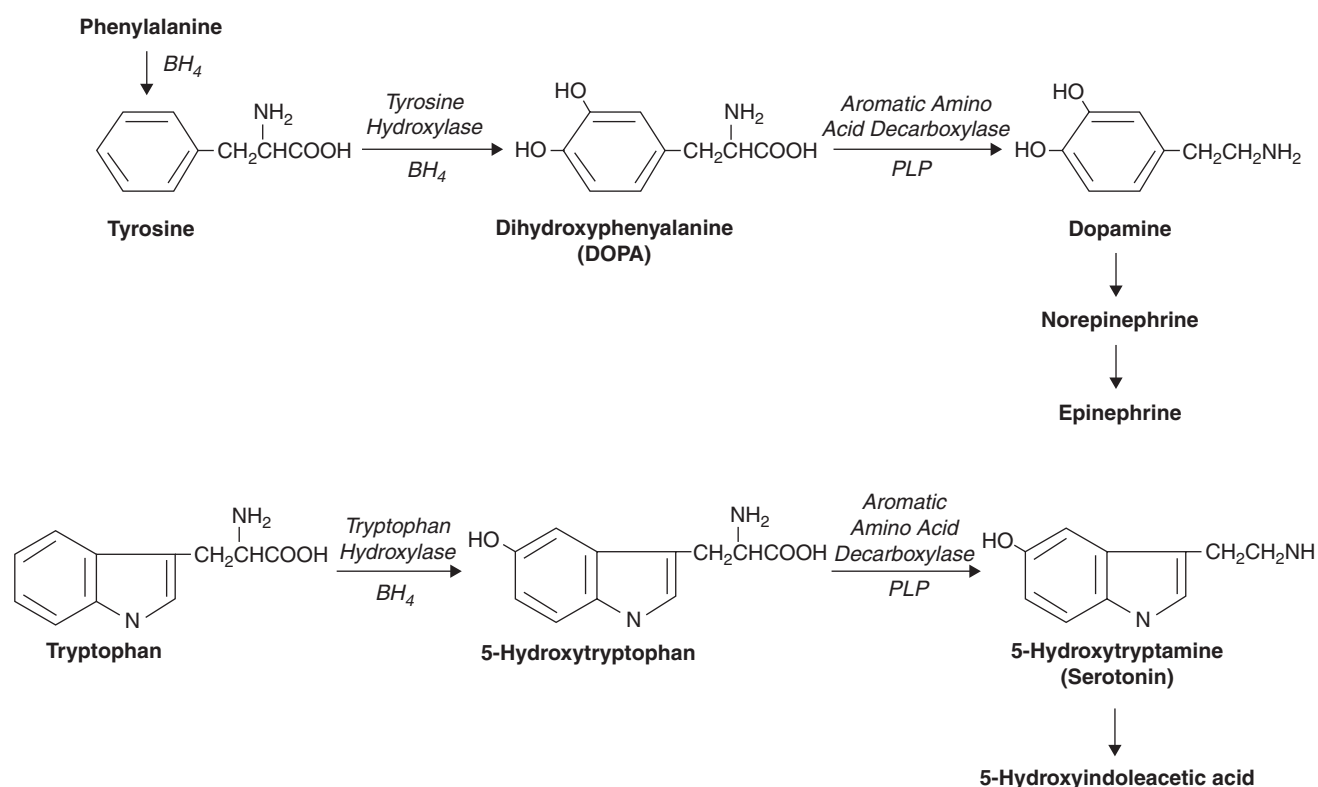


Figure 17.1 Pathways of neurotransmitter synthesis and modifications. The key enzymes illustrated are tyrosine hydroxylase and aromatic amino acid decarboxylase (AADC).

17.2, 17.3, 17.4, and 17.5). Impaired mental or motor development, or both, is also a uniform feature of the disease (63 percent) [3]. Autonomic manifestations include excessive sweating, temperature instability, nasal congestion, and hypersalivation. Patients have difficulty swallowing or eating. Speech is dysarthric and difficult. Dystonia may be accompanied by athetosis or chorea. Eye fixation is poor and 39 percent have had ptosis [3].



Figure 17.2 Boy with aromatic amino acid decarboxylase deficiency. He had severe truncal hypotonia and rigidity of limbs, as well as oculogyric crises. (Illustration was kindly provided by Dr Georg Hoffmann of Universitat Heidelberg.)

Irritability and insomnia are common. Head control is poor in infancy. Physical examination may reveal brisk knee jerks and extensor plantar responses [6].

Neonatal manifestations, in addition to truncal hypotonia and ptosis, include feeding problems, lethargy, hypothermia, and hypotension [7]. Intestinal manifestations include reflux, diarrhea, and constipation. Voluntary movements are difficult.

The diagnosis is dependent on the assessment of neurotransmitters and their metabolites in the CSF. It is



Figure 17.3 An adolescent with a special form of aromatic amino acid decarboxylase deficiency, i.e. L-dopa responsiveness. He was dystonic and had oculogyric crises. (Illustration was kindly provided by Dr Georg Hoffmann of Universitat Heidelberg.)



Figure 17.4 The patient shown in Figure 17.3 after treatment with L-dopa. (Illustration was kindly provided by Dr Georg Hoffmann of Universitat Heidelberg.)

necessary to follow a strict protocol for the collection of samples, with special tubes and prompt shipment to the referral laboratory overnight on dry ice [8–10]. Age-related reference values are critical [10], as well as recognition of the rostrocaudal pattern of concentrations.

Patterns of neurotransmitter metabolites in the CSF in this and other disorders of neurotransmitter metabolism are shown in Table 17.1. In patients with AADC deficiency, levels of HVA, HIAA, and MHTG are low. Levels of 3-O-methyldopa are markedly elevated. Levels of L-DOPA and 5-HT are elevated, but less so. This pattern was found in 100 percent of patients [3]. Levels of vanillic acid are elevated in the urine, and this may be the first clue as to the diagnosis as it is found on organic acid analysis of the urine. On the other hand, its elevation may be mild [3]. This



Figure 17.5 A girl with tyrosine hydroxylase deficiency displaying an oculogyric crisis. Reproduced from Hoffmann G.F., Assman B., Bräutigam C., Dionisis-Vici C., Häussler M., de Klerk J., Naunann M., Steenbergen-Spanjers G., Strassburg H.M., and Wevers R.A., (2003), Tyrosine hydroxylase deficiency causes progressive encephalopathy and dopa-nonresponsive dystonia. *Annals of Neurology* 54: 556–65, with permission from Wiley.

compound is formed from transamination of accumulated 3-O-methyldopa. The differential diagnosis of this pattern includes deficiency of pyridox(am)ine 5'-phosphate oxidase (PNPO), because this enzyme is responsible for the maintenance of pyridoxal phosphate (PLP) levels in the central nervous system (CNS). Mutations in the PNPO gene for this enzyme lead to deficiency of PLP and the same patterns of CSF metabolites as in AADC deficiency [11]. PLP is the cofactor for the AADC enzyme. Measurement of PLP in the CSF will distinguish AADC deficiency from PNPO deficiency.

Prolactin secretion by the pituitary is regulated by neurosecretory dopamine neurons in the hypothalamus. Dopamine inhibits secretion, so measurement of an increased serum prolactin may provide a clue to the diagnosis.

Table 17.1 Patterns of metabolites in the cerebrospinal fluid in patients with disorders of neurotransmitter metabolism

	Homovanillic acid (HVA)	5-Hydroxyindoleacetic acid (HIAA)	3-Methoxy-4-hydroxyphenylglycol (MHPG)	3-O-Methyldopa
Aromatic L-amino acid decarboxylase (AADC) deficiency	↓	↓	↓	↑
Tyrosine hydroxylase (TH) deficiency	↓	N	N	N
Disorders of BH ₄ synthesis (recessive)	↓	↓	↓	N
DOPA recessive dystonia-GTP hydrolase deficiency (dominant)	↓	N	N	N
Pyridoxine phosphate oxidase (PNPO)	↓	↓	↓	↑

Imaging of the brain and the EEG have been normal in most patients [3]. EEG findings of slow or fast waves and polyspikes have been seen. Some patients are found on magnetic resonance imaging (MRI) to have cerebral atrophy or degenerative changes in the white matter.

GENETIC AND PATHOGENESIS

The disease is inherited in an autosomal recessive fashion. Consanguinity has been observed [1, 6]. If the mutation can be detected, this would provide an ideal method for prenatal diagnosis and heterozygote detection.

Recently discovered missense mutations include p.L38P, p.Y79C, p.A110Q, p.G123R, and p.R412W [3]. Two frameshift mutations were p.I42fs and p.I433fs. So far genotype–phenotype correlations have been elusive.

In all patients in whom activity of the AADC enzyme in plasma has been measured, the values were very low or undetectable [3]. The diagnosis has also been confirmed by assay of enzyme activity in biopsied liver [1, 2]. Activities against each substrate were decreased to the same degree [11].

TREATMENT

Striking improvement in tone and mobility was reported [1, 2] in the twins initially reported following treatment with a monoamine oxidase inhibitor, a dopamine antagonist, and pyridoxine. Hyperdopaminuria found

in these patients increases with treatment with DOPA [12]. Treatment programs usually include pyridoxine, dopamine agonists, and monoamine oxidase inhibitors [5]. A suggestion of sex differences was found [5] in a series of five male patients who responded well to treatment and progressed developmentally and five females and two males who responded poorly. In a majority of patients recently summarized [3, 13], little or no benefit was observed.

Pyridoxine has been used in doses from 40 to 1800 mg/day (4–81 mg/kg). An excellent response to a monoamine oxidase inhibitor and a dopamine agonist was reported in two siblings with an ‘unusually mild phenotype’ [14]. Treatment with the immediate precursors L-DOPA and 5-hydroxytryptophan has generally been ineffective [3]. Nevertheless, three siblings responded dramatically to L-DOPA [15]. [Figures 17.3](#) and [17.4](#) illustrate such a response. First choice medications recommended [3] were dopamine agonists, such as pergolide or bromocriptine in combination with pyridoxine, and a monoamine oxidase inhibitor, such as selegiline. Bromocriptine is usually begun at a dose of 0.25 mg/kg per day. Pergolide is given at a very low starting dose of 0.006 mg/kg twice daily. Trihexyphenidyl has been begun at a dose of 0.03 mg/kg divided into three doses; the dose is then increased each week until improvement or until a dose of 0.5 mg/kg per day. It is recommended that doses over 15 mg be viewed with caution.

Folinic acid is recommended in doses of 10–20 mg/day, because of the possibility of cerebral folate depletion resulting from methylation of accumulated L-dopa.

Tyrosine hydroxylase deficiency

MAJOR PHENOTYPIC EXPRESSIONS

Deficiency of tyrosine hydroxylase is the cause of autosomal recessive Segawa syndrome. Autosomal dominant Segawa syndrome is caused by mutation in the guanosinetriphosphate cyclohydrolase (*GCH1*) gene ([Chapter 16](#)). Dopa-responsive dystonia (Segawa syndrome), hypokinetic rigidity, oculogyric crises, in type A; complex early onset encephalopathy including mental impairment in type B; low CSF HVA and MHPG; and deficiency of tyrosine hydroxylase.

INTRODUCTION

In 1996, Ludecke and colleagues [16] reported a patient with what they called recessively inherited L-DOPA responsive parkinsonism in infancy. Onset was at three months with involuntary jerky movements followed by generalized rigidity and few spontaneous movements. By six months, the face was expressionless and tongue tremulous. Drooling was prominent and there was bilateral ptosis. Cogwheel rigidity was noted. The dopamine metabolite HVA was present in very low concentrations in the CSF. Ptosis was improved by the ocular instillation of phenylephrine. Treatment with L-DOPA and carbidopa led to increase to normal of the CSF HVA and dramatic improvement in hypokinesia and the other parkinson features.

Marked improvement in response to treatment with L-DOPA was also reported in four Dutch patients with impaired motor development, hypokinesia, truncal hypotonia, masked facies, and rigidity of limbs [17, 18].

A different, more severe presentation was reported [19] in an Italian infant with onset at birth of progressive hypotonia, hypokinesia, dysphagia, extensive sweating, and irritability. He had dysphagia and reduced facial expression. Movements were dystonic. Concentration of HVA in the CSF was undetectable. Response to L-DOPA was limited.

In a study of 36 patients and review of the literature [20], distinction was made of the more common infantile onset DOPA-responsive phenotype as type A and the neonatal onset DOPA-nonresponsive type B. The authors recognized that there was likely a spectrum of phenotypes

between the two types. They pointed out a patient reported as an example of type A who had many of the features of type B [21]. Virtually all patients had onset by one year of age, the few exceptions by five years [20].

Tyrosine hydroxylase catalyzes the rate-limiting step in the formation of the catecholamines dopamine, norepinephrine, and epinephrine (Figure 17.1). Deficient activity of the enzyme leads to diagnostic decrease in CSF concentrations of the metabolic catecholamine degradation products, HVA and MPHG (Table 17.1). No patients had symptoms of extracerebral deficiency of catecholamines, such as abnormalities in maintenance of blood profile.

The gene TH contains 14 exons and has an open reading frame of 1491 bp [22]. It was mapped to chromosome 11p.15.5, the most distant end of 11p [23–25].

In the initial family with two affected siblings, mutation was found in exon 11 that led to p.Q412K [26]. In the fewer than 40 patients reported worldwide, mutations were almost exclusively missense [20]. This led to the conclusion that more severe disruptions of the gene may not be compatible with life.

CLINICAL PICTURE

Deficiency of tyrosine hydroxylase leads to clinical manifestations very early in life, nearly all by the first year birthday [20]. The clinical features are such that the diagnosis is usually not made clinically, but usually by the pattern of metabolites in the CSF (Table 17.1), so lumbar puncture is an essential step in elucidating the diagnosis. This disease and GTP cyclohydrolase are known as the DOPA-responsive dystonias. They are distinguished by the study of pterin metabolite in the CSF. Of the two, manifestations of tyrosine hydroxylase are in general much more severe.

Hypokinesia, bradykinesia, and dystonia are associated with a picture of infantile parkinsonism. Oculogyric crises are memorable features. These are the features of cerebral catecholamine deficiency [20, 21].

Neuroimaging was normal in the majority of patients, in 20 of 29 studied [20]. Nonspecific mild changes in white matter signaling were found in 19 percent of type A and 63 percent of type B patients. Gross abnormality of structure or signal intensity were never seen in the MRI.

The pattern of catecholamines in the CSF is distinctive (Table 17.1). Absence of elevation of HIAA distinguishes it from AADC deficiency. The HIV/HIAA ratio is useful, and it correlates well with the degree of clinical severity.

Prolactin concentration may be elevated in the serum, as in AADC deficiency. In one patient galactorrhea was described [27]; this result of hyperprolactinemia, a consequence of dopamine deficiency, was present before the development of neurologic features of the disease.

GENETICS AND PATHOGENS

The disease results from mutation in the tyrosine hydroxylase gene and is transmitted in an autosomal recessive fashion. The deficiency of the enzyme has seldom been documented, because of a conceptualization that there might be no expression in tissues outside the brain. So, most documentation has been via mutational analysis. In the original family with the Q412K mutation, the enzyme was demonstrated to have lowered affinity for tyrosine and residual activity of about 15 percent of control [28]. The most common mutations were c.698G>A and c.707T>C [20]. Genotype–phenotype correlations were not evident in these two.

Some 37 different mutations have resulted [20]. Of 100 alleles, 96 were affected by missense mutations leading to amino acid substitutions. Pathogenic mutations have been identified in the promoter region of the TH gene [29, 30]. The c.698G>A mutation in the Dutch population appears to have resulted from a founder effect [31]. Except for this, c.7059T>C and c.70G>A, all the others have been private mutations.

Only four patients have had stop codons leading to truncation of the protein, and no patient has had homozygosity or compound heterozygosity for two of these. The patients with the promoter mutations had type A phenotypes.

TREATMENT

Treatment of the central deficiency of dopamine with L-DOPA has been the treatment of choice, and virtually always with carbidopa [20]. The most readily available preparations contain carbidopa or benserazide. Doses of DOPA employed have been 3–10 mg/kg per day, but it is advisable to start with 0.5–1 mg/kg divided into three or four doses, and to increase weekly. Hypersensitivity to DOPA has been observed in type B patients; some tolerated only 0.5 mg/kg and some none. Inhibitors of dopamine degradation, such as selegiline, have been employed. Patients who tolerate a reasonable dose of L-DOPA generally displayed a good or moderately good response. Improvement in movement with doses of hypokinesia, tremor, rigidity, and dystonia have been dramatic, permitting impressive improvement in motor function. Children who had been wheelchair-bound for years have walked [20]. Most patients have not had CSF levels of HVA monitored, but improvement clinically was found, despite a failure to return CSF HVA to normal.

Tardive dyskinesia is a recognized side effect of treatment, usually responsive to a reduction in dosage.

Moderate mental impairment was judged to occur in follow up in 33 percent of type A patients and 91 percent of type B [20].

Dihydrofolate reductase deficiency

MAJOR PHENOTYPIC EXPRESSION

Megaloblastic anemia, seizures, developmental delay, cerebral folate deficiency and tetrahydrobiopterin deficiency, and deficient activity of dihydrofolate reductase.

INTRODUCTION

Deficiency of dihydrofolate reductase (DHFR) leads to depletion of cerebral tetrahydrobiopterin (BH4) which interferes with the formation of dopamine, norepinephrine, and serotonin. Patients with DHFR deficiency have megaloblastic anemia or pancytopenia and severe cerebral folate deficiency. Concentrations of cerebrospinal fluid 5-methyltetrahydrofolate (5-MTHF) and BH4 are markedly decreased. Cerebral manifestations included seizures, profound developmental delay, and cerebral atrophy. Treatment with folinic acid reverses hematologic manifestations.

Activity of DHFR is markedly reduced. In the first family described [32], consanguineous British Pakistanis, a homozygous missense mutation c.238 C>T (p.L80F) was found in exon 3. In another consanguineous European family, a homozygous c.458A>T (p.D153V) mutation was found [33]. The gene is localized in chromosome 5.

CLINICAL ABNORMALITIES

Clinical experience has been recorded in two back-to-back reports [32, 33] in 2011. The first patient [32] presented at four months of age with pallor, poor feeding, and microcephaly. Head circumference, normal at birth, was 36.8 cm, below the 0.4th percentile.

Blood smear revealed oval macrocytes and microcytes and hypersegmented neutrophils. He became pancytopenic, but the marrow revealed megaloblastic erythropoiesis. Levels of folate, B₁₂, and ferritin were normal, and he was thought to have transcobalamin II deficiency, but there was no response to hydroxycobalamin.

Within a week, he began having generalized tonic-clonic and right-side focal seizures unresponsive to phenytoin, benzodiazepines, and pyridoxine. Phenobarbitone and levetiracetam yielded partial control. MRI was that of severe cerebral and even more cerebellar atrophy.

A previous sibling had died at 28 weeks of Klebsiella pneumonia after developing anemia and intractable seizures. Autopsy revealed cerebral and cerebellar atrophy with loss of the internal and external granular layers. Neuronal and vascular calcifications were seen in the basal ganglia and subcortical white matter.

In a more attenuated phenotype, the proband presented at 11 years of age with macrocytosis, without anemia, and slow waves on EEG without epilepsy-specific potentials.

His brother presented at five years with anemia (5.6 g/dL), reticulocytosis, and a megaloblastic marrow. Serum folate, cobalamin, transcobalamin I and II binding capacity, and homocysteine were normal, as were urinary methylmalonate, orotate, and formiminoglutamate. Treatment with formiminoglutamate, folinic acid, and hydroxycobalamin reversed the hematologic findings. By eight years of age, he was found to have increasing learning difficulties and episodes of involuntary blinking or winking with impairment of consciousness. EEG was that of absence epilepsy.

The third sibling had a complicated febrile seizure at two years of age. Her erythrocytes were macrocytic at MCV109, but there was no anemia. At five years, she began to have episodes of blinking and impaired vision and consciousness. EEG was that of absence epilepsy and eyelid myoclonus. The marrow was megaloblastic.

Levels of 5-MTHF in the CSF were very low in patient 1 [33] and undetectable in patients 2 and 3, despite the fact that by the time of analysis they were being treated with folinic acid. In this family, irregular compliance with folinic acid treatment was followed by recurrent seizures. In the proband of the other family [1], CSF 5-MTHF was very low (9 nmol/L) and rose to normal with treatment with folinic acid. His CSF level of BH4 was 13 and 23 nmol/mL (normal level, 27–105). Plasma concentrations of phenylalanine and homocysteine were normal. CSF levels of homovanillic acid and 5-hydroxyindolacetic acid were slightly low, and they did not improve with folinic acid treatment.

GENETICS AND PATHOGENESIS

Both mutations described were missense mutations. The p.L80F was found in two British Pakistani patients. Modeling suggested destabilization of the protein or disruption of binding to NADPH [32]. The p.D153V appeared to influence the stabilization by D153 of the FG loop of DHFR [33].

DHFR catalyzes the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate. This step is essential for the synthesis of precursors of DNA synthesis, including glycine and purines, and the conversion of deoxythymadinemonophosphate to deoxyuridine-monophosphate. It is also the only enzyme catalyzing the reduction of folic acid to dihydrofolate. It is a key enzyme present in all dividing cells. It is the target of methotrexate

in cancer chemotherapy and of trimethoprim (antibiotic) and pyrimethamine (antiprotozoal), all of which inhibit DHFR.

Activity of DHFR in lymphoblasts derived from the proband was 1.5 nmol/THF/hour per mg protein. The mother and father were 59.7 and 52.9, while controls approximated 150 [32]. In a different assay [33], activity in patient lymphoblasts was 10 percent of control. Binding of fluorescein-labeled methotrexate to lymphoblastoid cells was severely reduced [33]. Immunoblotting revealed reduced protein expression [33].

Deficiency of cerebral BH4 was not corrected by folinic acid. The absence of hyperphenylalaninemia indicated that this deficiency was restricted to the central nervous system. This cofactor for tyrosine hydroxylase and tryptophan hydroxylase is essential for the formation of dopamine, norepinephrine, and serotonin.

TREATMENT

Hematologic manifestations of the disease are readily reversed by treatment with folinic acid. Doses employed have ranged from 5 to 30 mg/day [32,33] to 1 mg/kg per day.

Following treatment, CSF 5-MTHF became normal [33]. One patient became independent of anticonvulsant medication [2]. Improvement in school performance was also recorded [33].

REFERENCES

- Hyland K, Clayton PT. Aromatic amino acid decarboxylase deficiency in twins. *J Inherit Metab Dis* 1990; **13**: 301
- Hyland K, Surtees RAH, Rodeck C *et al*. Aromatic L-amino acid decarboxylase deficiency: clinical features, diagnosis, and treatment of a new inborn error of neurotransmitter amine synthesis. *Neurology* 1992; **42**: 1980
- Brun L, Ngu LH, Keng WT *et al*. Clinical and biochemical features of aromatic L-amino acid decarboxylase deficiency. *Neurology* 2010; **75**: 64.
- Chang YT, Mues G, McPherson *et al*. Mutations in the human aromatic L-amino acid decarboxylase gene. *J Inherit Metab Dis* 1998; **21**: 4.
- Pons R, Ford B, Chiribog CA *et al*. Aromatic L-amino acid decarboxylase deficiency: clinical features, treatment, and prognosis. *Neurology* 2004; **62**: 1058
- Maller A, Hyland K, Milstien S *et al*. Aromatic L-amino acid decarboxylase deficiency: clinical features, diagnosis, and treatment of a second family. *J Child Neurol* 1997; **12**: 349.
- Swoboda KJ, Saul JP, McKenna CE *et al*. Aromatic L-amino acid decarboxylase deficiency: overview of clinical features and outcomes. *Ann Neurol* 2003; **54**: 549.
- Hyland K. The lumbar puncture for diagnosis of pediatric neurotransmitter disease. *Ann Neurol* 2003; **54**(Suppl. 16): S13.
- Blau N, Duran M, Gibson KM (eds). *Laboratory Guide to the Methods in Biochemical Genetics*. Berlin: Springer-Verlag, 2008.
- Hyland K. Clinical utility of monoamine neurotransmitter metabolite analysis in cerebrospinal fluid. *Clin Chem* 2008; **54**: 633.
- Mills PB, Surtees RA, Champion MP *et al*. Neonatal epileptic encephalopathy caused by mutations in the PNPO gene encoding pyridox(am)ine 5'-phosphate oxidase. *Hum Mol Genet* 2005; **14**: 1007.
- Abeling NG, Brautigam C, Hoffmann GF *et al*. Pathobiochemical implications of hyperdopaminuria in patients with aromatic L-amino acid decarboxylase deficiency. *J Inherit Metab Dis* 2000; **4**: 325.
- Allen GF, Land JM, Heales SJ. A new perspective on the treatment of aromatic L-amino acid decarboxylase deficiency. *Mol Genet Metab* 2009; **97**: 6.
- Tay SK, Poh KS, Hyland K *et al*. Unusually mild phenotype of AADC deficiency in 2 siblings. *Mol Genet Metab* 2007; **91**: 374.
- Swoboda KJ, Hyland K, Goldstein DS *et al*. Clinical and therapeutic observations in aromatic L-amino acid decarboxylase deficiency. *Neurology* 1999; **53**: 1205.
- Ludecke B, Knappskog PM, Clayton PT *et al*. Recessively inherited L-DOPA responsive parkinsonism in infancy caused by a point mutation (L205P) in the tyrosine hydroxylase gene. *Hum Genet* 1996; **5**: 1023.
- Brautigam C, Wavers RA, Jansen RJT *et al*. Biochemical hallmarks of tyrosine hydroxylase deficiency. *Clin Chem* 1998; **44**: 1897.
- Weavers RA, de Rijk-van Andel JF, Brautigam C *et al*. A review of biochemical and molecular genetic aspects of tyrosine hydroxylase deficiency including a novel mutation (291delC). *J Inherit Metab Dis* 1999; **22**: 364.
- Brautigam C, Steenbergen-Spanjers GCH, Hoffmann GF *et al*. Biochemical and molecular genetic characteristics of the severe form of tyrosine hydroxylase deficiency. *Clin Chem* 1999; **45**: 2073.
- Willemsen MA, Verbeek MM, Kamsteeg EJ *et al*. Tyrosine hydroxylase deficiency: a treatable disorder of brain catecholamine biosynthesis. *Brain* 2010; **133**: 1810.
- Grattan-Smith PJ, Wevers RA, Steenbergen-Spanjers GC *et al*. Tyrosine hydroxylase deficiency: clinical manifestations of catecholamine insufficiency in infancy. *Mov Disord* 2002; **17**: 354.
- Nagatsu T, Ichinose H. Comparative studies on the structure of human tyrosine hydroxylase with those of the enzyme of various mammals. *Comp Biochem Physiol C* 1991; **98**: 203.
- Craig SP, Buckle VJ, Craig IW *et al*. Localization of the human tyrosine hydroxylase gene to chromosome 11p15. *Cytogenet Cell Genet* 1985; **40**: 610.
- Craig SP, Buckle VJ, Lamouroux A *et al*. Localization of the human tyrosine hydroxylase gene to 11p15: gene duplication and evolution of metabolic pathways. *Cytogenet Cell Genet* 1986; **42**: 29.
- Xue F, Kidd JR, Pakstis AJ *et al*. Tyrosine hydroxylase maps to the short arm of chromosome 11 proximal to the insulin and HRAS1 loci. *Genomics* 1998; **2**: 288.
- van den Heuvel LP, Luiten B, Smeitink J *et al*. A common point mutation in the tyrosine hydroxylase gene in autosomal recessive L-DOPA responsive dystonia in the Dutch population. *Hum Genet* 1998; **102**: 644.

-
27. Yeung WL, Lam CW, Hui J *et al.* Galactorrhea – a strong clinical clue towards the diagnosis of neurotransmitter disease. *Brain Dev* 2006; **28**: 389.
 28. Knappskog PM, Flatmark T, Mallet J *et al.* Recessively inherited L-DOPA responsive dystonia caused by a point mutation (Q381K) in the tyrosine hydroxylase gene. *Hum Molec Genet* 1995; **4**: 1209.
 29. Verbeek MM, Steenbergen-Spanjers GCH, Willemsen MAAP *et al.* Mutations in the cyclic adenosine monophosphate response element of the tyrosine hydroxylase gene. *Ann Neurol* 2007; **62**: 422.
 30. Ribases M, Serrano M, Fernandez-Alvarez E *et al.* A homozygous tyrosine hydroxylase gene promoter mutation in a patient with dopa-responsive encephalopathy: clinical, biochemical and genetic analysis. *Mol Genet Metab* 2007; **92**: 274.
 31. Ludecke B, Dworniczak B, Bartholome K. A point mutation in the tyrosine hydroxylase gene associated with Segawa's syndrome. *Hum Genet* 1995; **95**: 123.
 32. Cario H, Smith DEC, Blom H *et al.* Dihydrofolate reductase deficiency due to a homozygous DHFR mutation causes megaloblastic anemia and cerebral folate deficiency leading to severe neurologic disease. *Am J Hum Genet* 2011; **88**: 226.
 33. Banka S, Blom HJ, Walter J *et al.* Identification and characterization of an inborn error of metabolism caused by dihydrofolate reductase deficiency. *Am J Hum Genet* 2011; **88**: 216.

Homocystinuria

Introduction	144	Treatment	149
Clinical abnormalities	145	References	150
Genetics and pathogenesis	148		

MAJOR PHENOTYPIC EXPRESSION

Ectopia lentis, vascular occlusive disease, osteoporosis, accumulation of homocystine and methionine, and defective activity of cystathionine synthase.

INTRODUCTION

Homocystinuria was first described in 1962 by Carson, Neill and colleagues [1, 2]. The enzymatic defect was identified by Mudd and colleagues [3], two years later. Since then, considerable experience has been developed which has defined the clinical phenotype, the abnormal biochemistry, and the natural history of the disease [4].

The molecular defect is in the enzyme cystathionine synthase (EC 4.2.1.22) (Figure 18.1). This enzyme is on the metabolic pathway for methionine, and patients may be recognized by an increase in the concentration of methionine in the blood. This property forms the basis

for the inclusion of homocystinuria in most programs of routine neonatal screening. In some patients, accumulation of methionine may give a prominent, unpleasant odor. The clinical picture regularly includes many features, like subluxation of the lenses of the eyes, which are characteristic of a disorder of connective tissue. Extreme variability of clinical presentation is a consequence of whether or not there are thrombotic events, and if so which areas of the body suffer infarction. Variability also results from the fact that there are two distinct populations of homocystinuric patients, one of which responds to treatment with pyridoxine and one that does not [4].

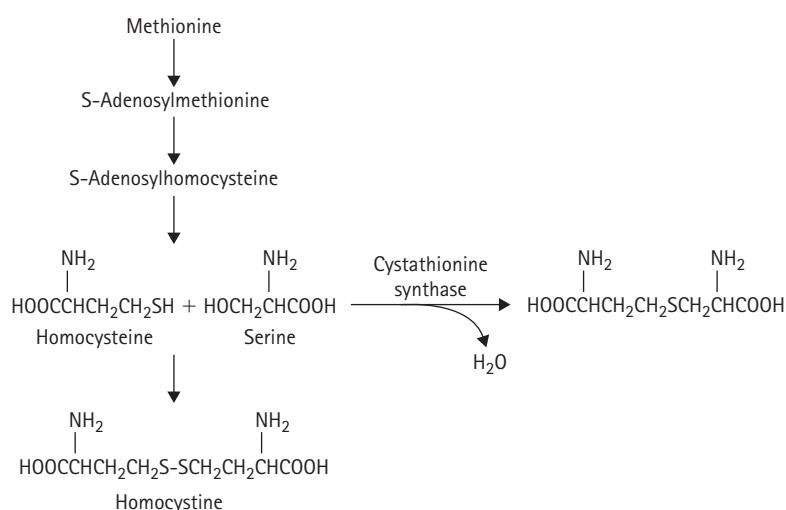


Figure 18.1 In homocystinuria, the defective enzyme is cystathionine synthase.



Figure 18.2 MG: A six-year-old boy with homocystinuria. He had short stature and genu valgum.



Figure 18.3 Closer view illustrates MG's eyes. Subluxed lenses had previously been removed bilaterally, after which he developed glaucoma in the left eye. He had fair skin and hair and a pronounced malar flush.

CLINICAL ABNORMALITIES

Ectopia lentis is a striking and readily recognizable manifestation of the disease (Figures 18.2, 18.3, 18.4, and 18.5). It may be the only manifestation [4–8] and, by 38 years of age, only 3 percent of patients have both lenses in

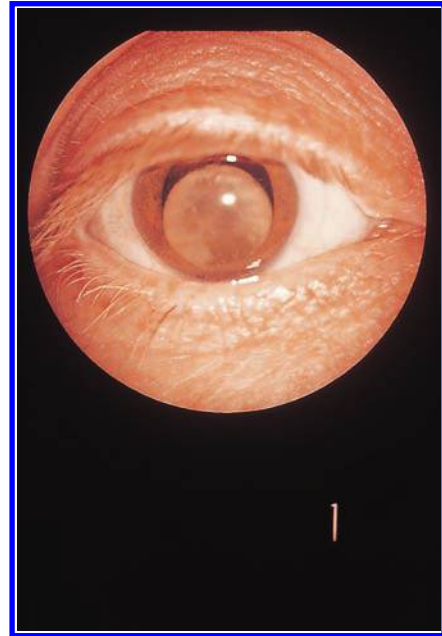


Figure 18.4 The dislocated lens in homocystinuria is usually downward, while in Marfan syndrome it is upward.



Figure 18.5 NMM: A ten-year-old girl with homocystinuria. She had been found one year previously to have left-sided glaucoma and subluxed lenses bilaterally. The left lens was removed.

place. Dislocation is usually present by ten years of age. The dislocation is said to be usually, but not always, downward – the opposite of the situation in Marfan disease. Its presence may be signaled by iridodonesis, a dancing or shimmering of the iris. Electron microscopy reveals partially broken zonules, abnormal zonular attachment, and a spongy capsular appearance [8]. Complications may include dislocation into the anterior chamber and papillary block glaucoma (Figure 18.3). Other ocular abnormalities include myopia, optic atrophy, cataracts, or retinal detachment [9].

The pigmentation of the iris may be lighter than in family members, and the same may be true for the skin and



Figure 18.6 Pronounced genu valgum in a three-year-old with homocystinuria.



Figure 18.8 NMM: She appeared tall and thin and height was in the fifth percentile. She had long, thin fingers.



Figure 18.7 Genu valgum in a patient with homocystinuria. The ankles were also in valgus and the feet everted.



Figure 18.9 A marfanoid appearance in a patient with homocystinuria. He had a prominent pectus carinatum and very thick corrective lenses.

hair. A pronounced malar flush [10] was first recognized in Ireland, but we have also seen it in patients with considerable cutaneous pigment (Figures 18.2 and 18.3). The skin may otherwise have blotchy erythema and pallor, and livido reticularis [11] is particularly common in the

distal extremities, which may be quite cold and show other evidence of vascular instability.

Skeletal abnormalities are prominent, especially genu valgum (Figure 18.6). Valgus may also be present in the ankles, often along with pes cavus. The feet may be everted (Figure 18.7). Some patients may be tall and thin and have a marfanoid appearance (Figures 18.8 and 18.9), but true arachnodactyly is rare, and some patients have a failure to thrive or shortness of stature. Pectus excavatum or carinatum may be present (Figure 18.9). There is a generalized osteoporosis. This is the most common musculoskeletal change and 50 percent of patients have osteoporosis by the end of the second decade. Roentgenograms (Figures 18.10, 18.11, and 18.12) characteristically reveal platyspondyly. There may be posterior biconcave or fish mouth appearance, and there may be impressive compression fractures or kyphoscoliosis [12, 13].

Impaired mental development is common but not invariable. This is probably a function of the presence or absence of thrombotic or vascular disease involving the



Figure 18.10 Roentgenogram of the hand of the patient shown in Figures 18.5 and 18.8 illustrates the arachnodactyly.

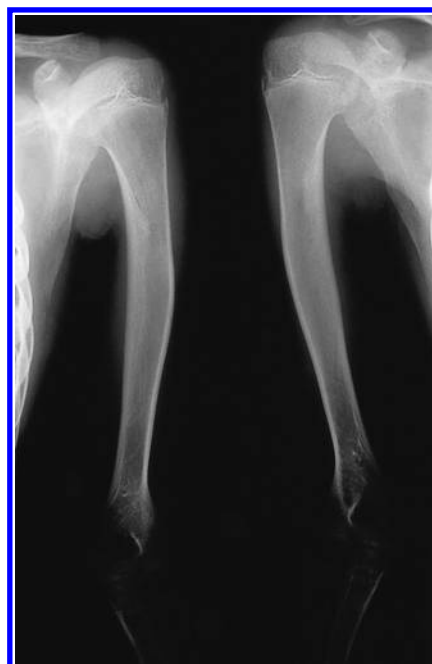


Figure 18.12 Roentgenogram of the humeri of a 12-year-old girl with homocystinuria revealed osteopenia and lateral bowing.

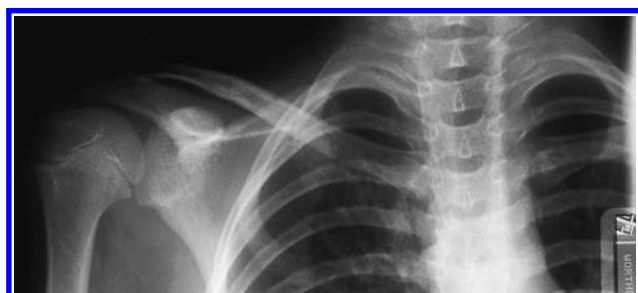


Figure 18.11 Roentgenogram of the spine of the same patient revealed osteopenia and compression of thoracic vertebrae.

nervous system. In patients responding to pyridoxine, the mean IQ was 78, while that of nonresponders was 64 [4]. IQ scores among affected siblings were similar [14]. Seizures occurring in about 20 percent of patients and abnormalities of the electroencephalogram (EEG), also common, probably also reflect the variable nature of vascular accident in this disease. Many patients have been observed to have typical strokes, with transient or permanent hemiplegia. A small number of patients has had dystonia [14–19]. Spasmodic torticollis may usher in ultimately fatal dystonia.

Psychiatric abnormalities have been observed in more than half of one series of 63 patients [14]. Three children were reported to have folate-responsive periodic behavior, including rage attacks [20, 21]. A three-year-old had episodic repetitive behavior thought to represent psychomotor seizures [20]. Adults have been diagnosed as schizophrenic or depressed [14], or to have personality disorders.



Figure 18.13 Prominent venous pattern in the feet of a man with homocystinuria.

Neuroimaging by computed tomography (CT) or magnetic resonance imaging (MRI) may be normal until the occurrence of cerebrovascular disease. Evidence of infarction has been obtained in patients presenting with hemiparesis, with or without papilledema. Cerebral venous and dural sinus thrombosis has been demonstrated by CT scan and confirmed by digital subtraction angiography [22].

Neuropathologic study has revealed occlusion of vessels, old and new thrombi, spongy degeneration, and neuronal loss [23–25]. In the patient who died at 18 years of age with dystonia, the brain was histologically normal [17].

Vascular disease in homocystinuria involves the vessels themselves, as well as a tendency of the blood to clot (Figure 18.13). Thromboses may be arterial or venous, and may be fatal. Cerebrovascular disease occurred in one-third of 147 patients, and 32 percent of those thromboembolic events were strokes [4]. Ten patients had myocardial infarction. There were 11 percent peripheral arterial occlusions, 51 percent peripheral venous occlusions, and 32 of these patients had pulmonary emboli. Surgery may be especially strongly associated with thromboembolic accidents [26]. Medial degeneration of the vessels and intimal proliferation both narrow vessel lumens, and initial injury is followed by the adherence of unusually sticky platelets. The end result is severe narrowing of the arteries. This may be demonstrated angiographically, as may aneurysmal dilatation [27].

Acute pancreatitis has been reported [28] in two patients, one of whom developed a large pseudocyst that was drained surgically. Spontaneous pneumothorax has been reported in three patients with this disease, one of whom had two episodes [29, 30].

Homocystinuria and pregnancy have been reported [31] in 11 women (15 pregnancies), six of them pyridoxine responsive and five nonresponsive. Complications of pregnancy included pre-eclampsia in two and a venous thrombosis in a leg in one. There were two spontaneous and one induced abortion. Of 12 live born infants, ten were normal and two had multiple anomalies, one of which was the Beckwith-Wiedemann syndrome, which were judged to be unrelated to homocystinuria.

GENETICS AND PATHOGENESIS

Deficiency of cystathionine synthase is autosomal recessively transmitted. It occurs with a frequency of one in 50,000 in Ireland and New England and one in one million in Japan; overall frequency is between one in 200,000 and 300,000. The defective enzyme may be demonstrated in cultured lymphocytes and fibroblasts, as well as in tissues such as liver. Ranges of activity are from zero to 10 percent of the control mean. Pyridoxine-responsive patients always have some residual activity, and increased activity of hepatic enzyme has been documented in response to treatment. Three types of enzyme were delineated: zero residual activity; reduced activity and normal affinity for pyridoxalphosphate; and reduced activity and affinity [32]. In a study of fibroblasts of 20 patients, each of 14 with residual enzyme activity had cross-reacting material (CRM) [33]; in six with undetectable activity, three had no CRM and three had reduced amounts of CRM.

The enzyme cystathionine β -synthase is a tetramer of 63 kDa [34], which undergoes post-translational proteolytic increase in activity with decrease in size to

48 kDa. It has binding sites for pyridoxal phosphate, as well as homocysteine and serine. S-adenosylmethionine and heme are activators [35, 36]. In most patients, enzyme size is normal, but exceptions have been encountered [37].

The locus for human cystathionine β -synthase was mapped to chromosome 21 by Chinese hamster-human cell hybrids [38], and cDNA prepared from immunopurified mRNA [39] was used to verify the locus at the subtelomeric region of chromosome 21q22.3 [40], where it is syntenic with α -A-crystallin. There are 23 exons over some 28 kb [41], from which the 551 amino acids are encoded by exons 1–14 and 16. Alternate splicing may include exon 15, which is represented in a few mRNA molecules, but its 14 encoded amino acids are not found in the expressed enzyme. There is also alternative splicing among five exons (designated -1α to -1ϵ) in the 5'-untranslated region. More than 140 mutations have been identified [42], and the functional consequences in many have been confirmed by expression systems. Among the first to be identified was a G to A change at 919 in exon 8, which converts glycine 307 to serine [43], and this mutation is the leading cause of homocystinuria in Ireland. This and the pyridoxine-responsive I278T were the most common of 310 homocystinuric alleles [44].

Another point mutation in exon 8 is a T to C transition at position 833, causing a substitution of threonine for isoleucine 278, which is the predominant mutation in the Netherlands and in Italy, and is associated with a pyridoxine-responsive phenotype when it is homozygous, and may or may not be when present in a compound heterozygote. Another frequent alteration is a splice mutation in intron 11, 1224-2 A > C (IVS 11-2 A > C), which results in the skipping of all of exon 12. Interestingly, about half of the point mutations in the coding region originate from deamination of methylcytosine in CpG dinucleotides [44], and nearly one-quarter of the point mutations are found in exon 3, the most highly conserved region of the gene.

Most patients have been compounds of two different mutant alleles and most mutations are private [44]. Among compounds, a pyridoxine-responsive patient had the I278T mutation, as well as a 135-bp deletion that deleted 45 amino acids from 408 to 453 [45]. This patient had been previously found to have one abnormally small polypeptide subunit [37]. An interesting mutation [46] in a pyridoxine-responsive patient homozygous for G1330A changed aspartate 444 to asparagine and abolished the regulatory stimulation of activity by S-adenosylmethionine. A general lack of correlation between genotype and phenotype is exemplified by three siblings with the same molecular defect, one of whom had a single episode of claudication in the calf as his only clinical manifestation, while the other two had marked defects in intellectual function and skeletal changes [47].

A novel type of mutation was reported [48] in patients with homocystinuria and premature thrombosis, but without ectopia lentis or any of the other abnormalities of connective tissue. These were p.I435T, p.Q422L, and

p.S466L, located in the noncatalytic terminal region of the synthase gene, coding for enzymes that were catalytically active, but lacking in response to S-adenosylmethionine. These observations raise the possibility that the structural abnormalities of this disease may not be caused by elevated levels of homocysteine.

Among 11 families in Georgia, USA, p.I278T and p.T353M amounted to 45 percent of mutated alleles [49]. The former was exclusively Caucasian and B₆-responsive, while the latter was exclusively Black-American, and unresponsive to B₆. In Denmark, most of those homozygous for p.I278T were either unaffected clinically or had only a thromboembolic event after the third decade [50]. None had skeletal or other connective tissue abnormalities.

Deficiency of the enzyme leads to the accumulation of homocysteine and its excretion in large amounts in the urine. Patients generally have elevated concentrations of methionine in blood, and newborn screening programs have been based upon blood methionine levels [51]. Screening for urinary homocysteine in the past was most readily carried out by using the nitroprusside tests (Figure 18.14) [52, 53], which are tests for the excretion of sulfur-containing amino acids, or by staining a paper chromatogram or electropherogram with iodoplatinate. The diagnosis can be confirmed by quantification of the amino acids of the urine, where homocysteine and the mixed disulfide of cysteine and homocysteine are found. Since the major portion of homocysteine in plasma is bound to protein [54], the preferred method is to determine total plasma homocyst[e]ine by adding a reducing agent to release bound homocysteine prior to deproteinization, after which high performance liquid chromatography (HPLC) with detection of a fluorescent thiol reagent [55] or mass spectrometry [56] may be used.

Heterozygosity has been documented by the assay of cystathionine synthase in lymphocytes, fibroblasts, and liver [3]. Prenatal diagnosis has been accomplished by assay of the enzyme in cultured amniocytes, and affected fetuses

have been detected [57, 58]. Activity in normal chorionic villus material is so low that prenatal diagnosis by this method is precluded. Neonatal screening programs depend predominantly on screening for elevated concentrations of methionine in blood. Clearly, they miss a certain number of patients, particularly those who are pyridoxine responsive and thus most amenable to therapy, but they do provide early diagnosis for some patients.

Heterozygote detection has been carried out by enzyme assay of cultured fibroblasts, but there is overlap between carrier and controls [59, 60]. As many as 90 percent of carriers have been estimated to be detectable by measuring peak plasma levels of homocysteine after an overload of methionine [61].

TREATMENT

Pyridoxine responsiveness should be determined in all patients with homocystinuria, and those who respond should be treated. This is the major feature currently determining prognosis [4]. Of six patients treated from the neonatal period, IQ scores ranged from 82 to 110 [4]. Evidence that early treatment inducing good control of levels of homocysteine (<11 $\mu\text{mol/L}$) prevents mental impairment was reported [62] in experience with 23 patients, 19 diagnosed by newborn screening. Of 13 compliant patients, mean IQ was 106, which matched that of ten controls, while those poorly compliant had a mean IQ of 81; two untreated patients had IQs of 52 and 53. It is clear that reduction of levels of homocysteine with pyridoxine will prevent thromboembolic events [4]; thromboembolic complications are decreased among those who respond to pyridoxine even in those treated late. Doses have ranged from 100 to 1200 mg/day and should be determined individually. Peripheral neuropathy has occurred in individuals treated with large doses of pyridoxine [63]. Doses up to 500 mg/day appear to be safe. A recent recommendation was for 300–600 mg/day [64]. Patients requiring larger doses to reduce levels of homocysteine should certainly be monitored with tests of nerve conduction. Folate deficiency should be avoided by concomitant treatment with folate. Dietary therapy is much less effective, but should be employed in B₆-unresponsive patients, especially in infancy where it is easiest to ensure compliance. A methionine-poor diet is usually supplemented with cysteine [4, 64]. Concentrations of homocyst[e]ine may also be reduced in B₆-unresponsive patients by treatment with betaine [65]. Doses have ranged from 86 to 280 mg/kg. Especially rigorous therapy is necessary to ensure minimal levels of homocysteine are maintained in preparation for surgery [4]. Vitamin C has been proposed to improve endothelial function [66]. Antiplatelet agents are used in patients with stroke to prevent recurrence [66].

Ancillary supportive measures may be necessary. Orthopedic intervention may be required for pes planus

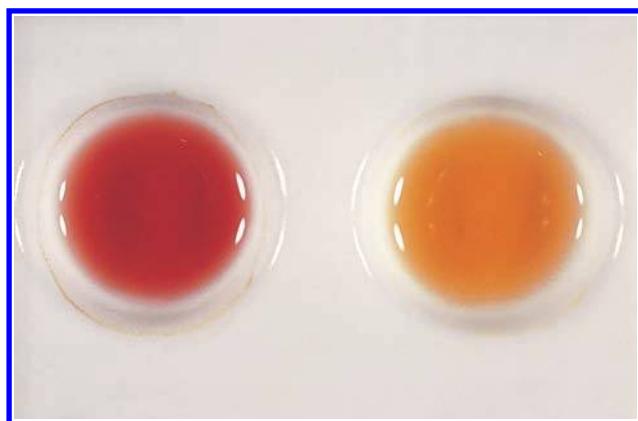


Figure 18.14 Positive cyanide nitroprusside test on the left indicating the excretion of larger than normal amounts of sulfhydryl-containing amino acid. The normal negative test is shown on the right.

and lower extremity valgus. The utility of agents such as bisphosphonates to increase bone mineralization remains to be established. Ectopia lentis may require aphakic contact lenses or spectacles; surgical intervention, such as lensectomy, may be indicated, and though there is controversy about the utility of implantation given the limited postoperative capsular support [67], intraocular lens implants may be considered.

REFERENCES

- Field CMB, Carson NAJ, Cusworth DC *et al*. Homocystinuria: a new disorder of metabolism. Tenth International Congress of Pediatrics 1962 (Abstr.).
- Carson NA, Neill DW. Metabolic abnormalities detected in a survey of mentally backward individuals in Northern Ireland. *Arch Dis Child* 1962; **37**: 505.
- Mudd SH, Finkelstein JD, Irreverre F, Laster L. Homocystinuria: an enzymatic defect. *Science* 1964; **143**: 1443.
- Mudd SH, Skovby F, Levy HL *et al*. The natural history of homocystinuria due to cystathionine beta-synthase deficiency. *Am J Hum Genet* 1985; **37**: 1.
- Spaeth GL, Barber GW. Homocystinuria. In a mentally retarded child and her normal cousin. *Trans Am Acad Ophthalmol Otolaryngol* 1965; **69**: 912.
- Drayer JI, Cleophas AJ, Trijbels JM *et al*. Symptoms, diagnostic pitfalls, and treatment of homocystinuria in seven adult patients. *Neth J Med* 1980; **23**: 89.
- Wilcken B, Turner G. Homocystinuria in New South Wales. *Arch Dis Child* 1978; **53**: 242.
- Michalski A, Leonard JV, Taylor DS. The eye and inherited metabolic disease: a review. *J Royal Soc Med* 1988; **81**: 286.
- Harrison DA, Mullaney PB, Mesfer SA *et al*. Management of ophthalmic complications of homocystinuria. *Ophthalmology* 1998; **105**: 1886.
- Carson NA, Cusworth DC, Dent CE *et al*. Homocystinuria: a new inborn error of metabolism associated with mental deficiency. *Arch Dis Child* 1963; **38**: 425.
- Gaull G, Sturman JA, Schaffner F. Homocystinuria due to cystathionine synthase deficiency: enzymatic and ultrastructural studies. *J Pediatr* 1974; **84**: 381.
- Schimke RN, McKusick VA, Huang T, Pollack AD. Homocystinuria. Studies of 20 families with 38 affected members. *J Am Med Assoc* 1965; **193**: 711.
- Brenton DP. Skeletal abnormalities in homocystinuria. *Postgrad Med J* 1977; **53**: 488.
- Abbott MH, Folstein SE, Abbey H, Pyeritz RE. Psychiatric manifestations of homocystinuria due to cystathionine beta-synthase deficiency: prevalence, natural history, and relationship to neurologic impairment and vitamin B6-responsiveness. *Am J Med Genet* 1987; **26**: 959.
- Hagberg B, Hambræus L, Bensh K. A case of homocystinuria with a dystonic neurological syndrome. *Neuropadiatrie* 1970; **1**: 337.
- Davous P, Rondot P. Homocystinuria and dystonia. *J Neurol Neurosurg Psychiatry* 1983; **46**: 283.
- Kempster PA, Brenton DP, Gale AN, Stern GM. Dystonia in homocystinuria. *J Neurol Neurosurg Psychiatry* 1988; **51**: 859.
- Arbour L, Rosenblatt B, Clow C, Wilson GN. Postoperative dystonia in a female patient with homocystinuria. *J Pediatr* 1988; **113**: 863.
- Berardelli A, Thompson PD, Zaccagnini M *et al*. Two sisters with generalized dystonia associated with homocystinuria. *Mov Disord* 1991; **6**: 163.
- Murphy JV, Thome LM, Michals K, Matalon R. Folic acid responsive rages, seizures and homocystinuria. *J Inherit Metab Dis* 1985; **8**(Suppl. 2): 109.
- Freeman JM, Finkelstein JD, Mudd SH. Folate-responsive homocystinuria and 'schizophrenia' A defect in methylation due to deficient 5,10-methylenetetrahydrofolate reductase activity. *N Engl J Med* 1975; **292**: 491.
- Schwab FJ, Peyster RG, Brill CB. CT of cerebral venous sinus thrombosis in a child with homocystinuria. *Pediatr Radiol* 1987; **17**: 244.
- Carson NA, Dent CE, Field CM, Gaull GE. Homocystinuria: clinical and pathological review of ten cases. *J Pediatr* 1965; **66**: 565.
- Dunn HG, Perry TL, Dolman CL. Homocystinuria. A recently discovered cause of mental defect and cerebrovascular thrombosis. *Neurology* 1966; **16**: 407.
- Chou SM, Waisman HA. Spongy degeneration of the central nervous system: case of homocystinuria. *Arch Pathol* 1965; **79**: 357.
- Jackson GM, Grisolia JS, Wolf PL *et al*. Postoperative thromboemboli in cystathionine beta-synthase deficiency. *Am Heart J* 1984; **108**: 627.
- Wicherink-Bol HF, Boers GH, Drayer JI, Rosenbusch G. Angiographic findings in homocystinuria. *Cardiovasc Intervent Radiol* 1983; **6**: 125.
- Collins JE, Brenton DP. Pancreatitis and homocystinuria. *J Inherit Metab Dis* 1990; **13**: 232.
- Cochran FB, Sweetman L, Schmidt K *et al*. Pyridoxine-unresponsive homocystinuria with an unusual clinical course. *Am J Med Genet* 1990; **35**: 519.
- Bass HN, LaGrave D, Mardach R *et al*. Spontaneous pneumothorax in association with pyridoxine-responsive homocystinuria. *J Inherit Metab Dis* 1997; **20**: 831.
- Levy HL, Vargas JE, Waisbren SE *et al*. Reproductive fitness in maternal homocystinuria due to cystathionine beta-synthase deficiency. *J Inherit Metab Dis* 2002; **25**: 299.
- Fowler B, Kraus J, Packman S, Rosenberg LE. Homocystinuria: evidence for three distinct classes of cystathionine beta-synthetase mutants in cultured fibroblasts. *J Clin Invest* 1978; **61**: 645.
- Skovby F. Homocystinuria: clinical, biochemical and genetic aspects of cystathionine beta-synthase and its deficiency in man. *Acta Paed Scand* 1985; **321**: 1.
- Skovby F, Kraus JP, Rosenberg LE. Biosynthesis and proteolytic activation of cystathionine beta-synthase in rat liver. *J Biol Chem* 1984; **259**: 588.
- Kery V, Bukovska G, Kraus JP. Transsulfuration depends on heme in addition to pyridoxal 59-phosphate. Cystathionine beta-synthase is a heme protein. *J Biol Chem* 1994; **269**: 25283.

36. Kery V, Elleder D, Kraus JP. Delta-aminolevulinate increases heme saturation and yield of human cystathionine beta-synthase expressed in *Escherichia coli*. *Arch Biochem Biophys* 1995; **316**: 24.
37. Skovby F, Kraus JP, Rosenberg LE. Homocystinuria: biogenesis of cystathionine beta-synthase subunits in cultured fibroblasts and in an *in vitro* translation system programmed with fibroblast messenger RNA. *Am J Hum Genet* 1984; **36**: 452.
38. Skovby F, Krassikoff N, Francke U. Assignment of the gene for cystathionine beta-synthase to human chromosome 21 in somatic cell hybrids. *Hum Genet* 1984; **65**: 291.
39. Kraus JP, Williamson CL, Firgaira FA *et al*. Cloning and screening with nanogram amounts of immunopurified mRNAs: cDNA cloning and chromosomal mapping of cystathionine beta-synthase and the beta subunit of propionyl-CoA carboxylase. *Proc Natl Acad Sci USA* 1986; **83**: 2047.
40. Munke M, Kraus JP, Ohura T, Francke U. The gene for cystathionine beta-synthase (CBS) maps to the subtelomeric region on human chromosome 21q and to proximal mouse chromosome 17. *Am J Hum Genet* 1988; **42**: 550.
41. Kraus JP, Oliveriusova J, Sokolova J *et al*. The human cystathionine beta-synthase (CBS) gene: complete sequence, alternative splicing, and polymorphisms. *Genomics* 1998; **52**: 312.
42. Kraus JP, Kozich V, Janosik M. Cystathionine beta-synthase internet mutation database, krausabwaiinpage. Last updated March 7, 2011. Available from: www.uchsc.edu/cbs/cbsdata/cbsmain.htm.
43. Gu Z, Ramesch V, Kozich V *et al*. Identification of a molecular genetic defect in homocystinuria due to cystathionine b-synthase deficiency. *Am J Hum Genet* 1991; **49**: 406.
44. Kraus JP, Janosik M, Kozich V *et al*. Cystathionine beta-synthase mutations in homocystinuria. *Hum Mutat* 1999; **13**: 362.
45. Kozich V, Kraus JP. Screening for mutations by expressing patient cDNA segments in *E. coli*: homocystinuria due to cystathionine beta-synthase deficiency. *Hum Mutat* 1992; **1**: 113.
46. Kluijtmans LA, Boers GH, Stevens EM *et al*. Defective cystathionine beta-synthase regulation by S-adenosylmethionine in a partially pyridoxine responsive homocystinuria patient. *J Clin Invest* 1996; **98**: 285.
47. de Franchis R, Kozich V, McInnes RR, Kraus JP. Identical genotypes in siblings with different homocystinuric phenotypes: identification of three mutations in cystathionine beta-synthase using an improved bacterial expression system. *Hum Mol Genet* 1994; **3**: 1103.
48. Maclean KN, Gaustadnes M, Oliveriusova JP. High homocysteine and thrombosis without connective tissue disorders are associated with a novel class of cystathionine beta-synthase (CBS) mutations. *Hum Mutat* 2002; **19**: 641.
49. Kruger WD, Cox DR. A yeast assay for functional detection of mutations in the human cystathionine beta-synthase gene. *Hum Mol Genet* 1995; **4**: 1155.
50. Skovby F, Gaustadnes M, Mudd SH. A revisit to the natural history of homocystinuria due to cystathionine beta-synthase deficiency. *Mol Genet Metab* 2010; **99**: 1.
51. Peterschmitt MJ, Simmons JR, Levy HL. Reduction of false negative results in screening of newborns for homocystinuria. *N Engl J Med* 1999; **341**: 1572.
52. Thuy LP, Nyhan WL. A screening method for cystine and homocystine in urine. *Clin Chim Acta* 1992; **211**: 175.
53. Spaeth GL, Barber GW. Prevalence of homocystinuria among the mentally retarded: evaluation of a specific screening test. *Pediatrics* 1967; **40**: 586.
54. Wiley VC, Dudman NP, Wilcken DE. Interrelations between plasma free and protein-bound homocysteine and cysteine in homocystinuria. *Metabolism* 1988; **37**: 191.
55. Jacobsen DW, Gatautis VJ, Green R. Determination of plasma homocysteine by high-performance liquid chromatography with fluorescence detection. *Anal Biochem* 1989; **178**: 208.
56. Magera MJ, Lacey JM, Casetta B, Rinaldo P. Method for the determination of total homocysteine in plasma and urine by stable isotope dilution and electrospray tandem mass spectrometry. *Clin Chem* 1999; **45**: 1517.
57. Fleisher LD, Longhi RC, Tallan HH *et al*. Homocystinuria: investigations of cystathionine synthase in cultured fetal cells and the prenatal determination of genetic status. *J Pediatr* 1974; **85**: 677.
58. Fowler B, Borresen AL, Boman N. Prenatal diagnosis of homocystinuria. *Lancet* 1982; **2**: 875.
59. Boers GH, Fowler B, Smals AG *et al*. Improved identification of heterozygotes for homocystinuria due to cystathionine synthase deficiency by the combination of methionine loading and enzyme determination in cultured fibroblasts. *Hum Genet* 1985; **69**: 164.
60. McGill JJ, Mettler G, Rosenblatt DS, Scriver CR. Detection of heterozygotes for recessive alleles. Homocyst[e]inemia: paradigm of pitfalls in phenotypes. *Am J Med Genet* 1990; **36**: 45.
61. Clarke R, Daly L, Robinson K *et al*. Hyperhomocysteinemia: an independent risk factor for vascular disease. *N Engl J Med* 1991; **324**: 1149.
62. Yap S, Rushe H, Howard PM *et al*. The intellectual abilities of early-treated individuals with pyridoxine-nonresponsive homocystinuria due to cystathionine beta-synthase deficiency. *J Inher Metab Dis* 2001; **24**: 437.
63. Bendich A, Cohen M. Vitamin B6 safety issues. *Ann NY Acad Sci* 1990; **585**: 321.
64. Testai FD, Gorelick PB. Inherited metabolic disorders and stroke part 2: homocystinuria, organic acidurias, and urea cycle disorders. *Arch Neurol* 2010; **67**: 148.
65. Wilcken DE, Wilcken B, Dudman NP, Tyrrell PA. Homocystinuria – the effects of betaine in the treatment of patients not responsive to pyridoxine. *N Engl J Med* 1983; **309**: 448.
66. Blum A, Hijazi I, Eizener MM *et al*. Homocysteine (Hcy) follow-up study. *Clin Invest Med* 2007; **30**: 21.
67. Neely DE, Plager DA. Management of ectopia lentis in children. *Ophthalmol Clin North Am* 2001; **14**: 493.

Maple syrup urine disease (branched-chain oxoaciduria)

Introduction	152	Treatment	158
Clinical abnormalities	153	References	160
Genetics and pathogenesis	156		

MAJOR PHENOTYPIC EXPRESSION

Overwhelming illness in the first days of life with lethargy progressive to coma, opisthotonus, and convulsions; recurrent episodes leading to developmental delay; characteristic maple syrup odor, branched-chain amino acidemia branched-chain oxoaciduria; deficiency of branched-chain oxoacid dehydrogenase.

INTRODUCTION

Maple syrup urine disease (MSUD) is a disorder of branched-chain amino acid metabolism in which elevated quantities of leucine, isoleucine, and valine and their corresponding oxoacids accumulate in body fluids [1]. The disease was first described in 1954 by Menkes and colleagues [2], who observed an unusual odor quite like that of maple syrup in the urine of four infants who died of a progressive encephalopathic disease in the first weeks of life. Elevated quantities of the branched-chain amino acids were found by Westall and colleagues [3], and the oxoacids derived from each of these amino acids were isolated and identified as their 2,4-dinitrophenylhydrazones by

Menkes [4]. Defective decarboxylation of ^{14}C -oxoacid was demonstrated in leukocytes by Dancis and colleagues [5].

The fundamental defect is in the activity of the branched-chain oxoacid dehydrogenase multienzyme complex (Figures 19.1 and 19.2) [1, 6, 7]. The components are E1 (a decarboxylase), E2 (an acyl transferase), and E3 (a flavoprotein lipoamide dehydrogenase (dihydrolipoyl dehydrogenase)). E1 is composed of two proteins in an $\alpha_2\beta_2$ structure. The enzyme complex, which was purified to homogeneity by Pettit and colleagues [7], is analogous to the pyruvate dehydrogenase complex (PDHC) (Chapter 49); in fact, the E3 component of the two complexes is the same protein, and in E3 deficiency (Chapter 50) defective activity of each dehydrogenase enzyme results. Expression

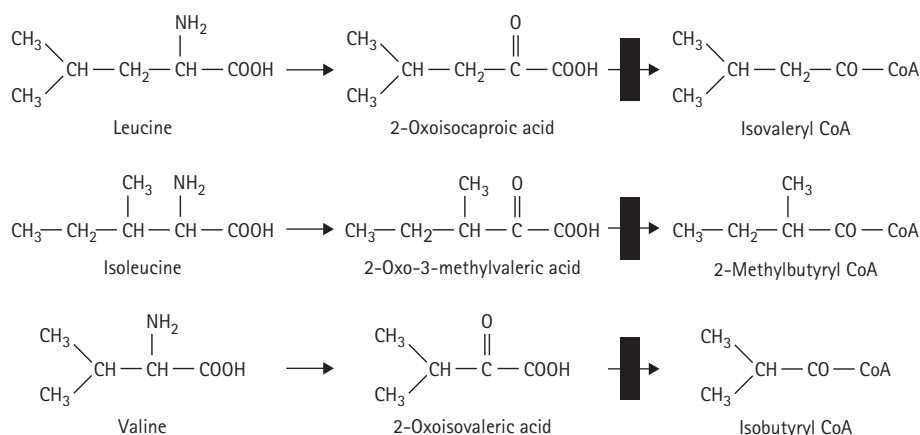


Figure 19.1 Metabolic pathways in the catabolism of leucine, isoleucine, and valine. The site of the defect is shown at the oxoacid step in each of the three pathways.

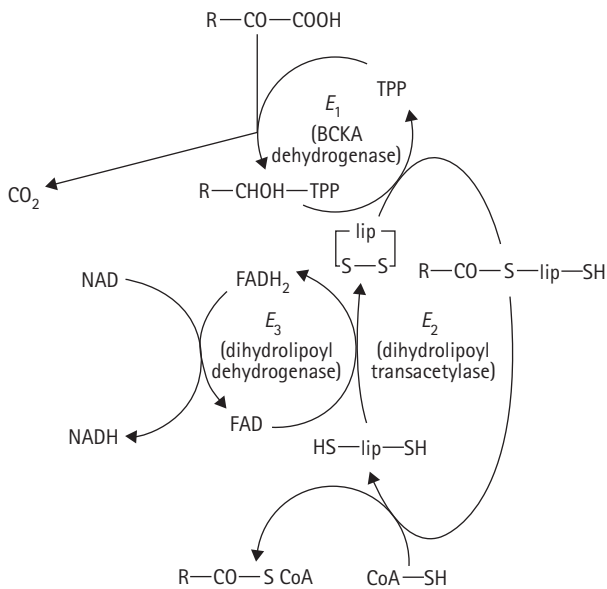


Figure 19.2 The branched-chain ketoacid dehydrogenase complex.

studies have shown that the complex does not assemble spontaneously; the $E1 \alpha$ and β proteins require chaperonins for folding and assembly [8].

The cDNA of each of the component genes has been cloned. The $E1\alpha$ gene has been localized to chromosome 19q13.1-13.2 [9], $E1\beta$ to 6p21-22 [10], $E2$ to 1p31 [10], and $E3$ to 7q31-32 [11]. Mutations have been identified in each gene. To date, a majority has been in the $E1\alpha$ and $E2$ genes. The mutation in the Mennonite population in which MSUD is common is a T to A transition that yields a single missense tyrosine to asparagine (Y393N) change at position 393 of the $E1\alpha$ gene [12]. In the Ashkenazi Jewish population, p.R183P mutation in the $E1\beta$ gene has been found in high frequency [13].

CLINICAL ABNORMALITIES

Infants with classic MSUD appear normal at birth, but they usually remain well for only a few days. Vomiting or difficulty to feed may be early symptoms. Usually by the end of the first week they become lethargic and progressive neurologic deterioration is rapid [14–16]. The cry may be high pitched. There may be periods of flaccidity, in which deep tendon reflexes and Moro reflex are absent, alternating with hypertonicity. General muscular rigidity is common. The absolutely characteristic picture is of a markedly comatose or semicomatose infant with hypertonia in an opisthotonic position (Figure 19.3). Extreme opisthotonus of this degree is very unusual in an infant only a few days old. There may be dystonic extension of the arms or a decerebrate appearance. Rarely, an infant may present with hypotonia and flaccidity (Figure 19.4). There may be



Figure 19.3 MB: An 11-day-old infant with maple syrup urine disease. He is shown in the characteristic opisthotonic position. (The illustration was kindly provided by Dr Havelock Thompson of the University of West Virginia.)



Figure 19.4 GV: A 3-week-old infant with maple syrup urine disease who presented with almost pure hypotonia. At this age, treatment had begun and tone improved somewhat, but he was still largely flaccid and had no sucking reflex. Tone and sucking became normal as the levels of the branched-chain amino acids were brought to normal.

abnormal eye movements. Convulsions occur regularly. These symptoms proceed to apnea, coma, and death, unless a vigorous therapeutic program is instituted [14]. Cerebral edema and a picture of pseudotumor cerebri may be seen and has been documented by computed tomography (CT) or magnetic resonance imaging (MRI) scan [17–20].

Rarely, an infant who is not effectively treated may survive this early phase of the disease and be left with prominent neurological abnormalities and severely impaired mental development (Figures 19.5, 19.6, 19.7, 19.8, and 19.9). Any patient with MSUD remains a candidate for further episodes of acute overwhelming illness and coma, any one of which may be fatal or lead to neurologic damage. Episodic illness is often triggered by the catabolic state that accompanies infection. It may also follow dietary indiscretion in which



Figure 19.5 A Saudi patient who was admitted in the first week of life with coma and convulsions, and was found to have maple syrup urine disease. He required ventilation, peritoneal dialysis, and parenteral nutrition. He spent four months in hospital and was discharged in good condition.



Figure 19.6 The same patient at two years of age. He had normal growth; however, his development was at the 18 month level.

the amount of protein ingested by normal infants or children are consumed. Cerebral edema and death have been reported in four infants between three and five years of age during therapy for severe metabolic acidosis and dehydration, which occurred with intercurrent infection



Figure 19.7 Mexican infant with maple syrup urine disease in relapse. She was semicomatose, had hypertonia, and had exaggerated deep tendon reflexes and ankle clonus.



Figure 19.8 ESH: A Saudi infant with maple syrup urine disease. He was quite rigid. The dermatitis reflects the problem of the dietary management of the disease.

[18]. Four patients with cerebral edema were documented to have hyponatremia and decreased osmolality in the serum [20].

The characteristic odor may be detected as soon as neurological symptoms develop. At the same time, it should be pointed out that not every patient with this disease is recognizable by the smell. Infants with this clinical picture should be screened for metabolic disease, whether or not an odor is detected. The odor is particularly likely to be absent in a comatose patient who has not received protein for days and has received copious amounts of parenteral fluid prior to transfer from a referring hospital. The odor may be found in the hair, the sweat, or cerumen. It is usually best appreciated in the urine. Freezing the urine may bring out the smell by concentrating it in an oil that freezes poorly or not at all at the top of the frozen specimen (Figure 19.10). The odor is sweet, malty, or caramel-like. It really does call forth an olfactory image of maple syrup. The odor of the patient with the disease once appeared provincially North American because of the localized occurrence of maple



Figure 19.9 CH: A teenager with maple syrup urine disease. She has severely impaired mental development and ataxic.



Figure 19.10 Frozen urine from a patient with untreated maple syrup urine disease. The odor of maple syrup is concentrated in an oil at the top.

syrup. Then Mediterraneans and others [21–23] realized that this odor was produced by the ingestion of fenugreek (*foenum graecum*) from *Trigonella* by an infant, or by the mother prior to delivery. The compound responsible for the odor was isolated and identified as sotolone (4,5-dimethyl-3-hydroxy-2[5H]-furanone). This compound, derived

from isoleucine, has now been isolated from the urine of patients with maple syrup urine disease, as well as from maple syrup itself [24].

Hypoglycemia may be observed in the acute episode of illness, but is not common [25, 26]. Acidosis is also not a major feature, although the patient may be ketonuric. Pancreatitis has been observed in MSUD, as it has in other organic acidemias [27, 28]. One patient with pancreatitis had transitory retinopathy [26].

The electroencephalogram (EEG) of the newborn with MSUD has been described as a comb-like rhythm [28, 29] of sharp waves. Later, the EEG may be normal between attacks, when there may be generalized slowing or paroxysmal discharges. A normal EEG may be seen despite abnormalities on CT of the brain and developmental delay [30]. Leucine loading in an asymptomatic adult with MSUD led to EEG abnormalities [31].

Neuroimaging most commonly shows decreased attenuation in white matter consistent with delayed or abnormal myelination [16, 29, 32, 33]. This appearance resolves after some months of successful treatment. In one patient [33], complete resolution of white matter lucency on CT of the brain was seen after 40 days of treatment. Impressively, ventricular size decreased. Generalized lucency of the cerebral white matter has been seen as early as 9 days, despite a restricted diet [34]. In nine of ten patients with classic MSUD who had general lucency, there was also localized intense lucency in the deep cerebellar white matter and peduncles and the brain stem. These changes have been attributed to edema [34], but may well be dysmyelination or delayed myelination [26, 35]. In one of our patients, MRI at one month showed striking lucency of the white matter which had markedly improved by one year of age. In another patient studied first by MRI of the brain at six years of age, in whom the initial diagnosis had been late, the MRI was normal, except for a slight increase in ventricular size.

The usual neuropathological finding in patients dying of MSUD is a generalized status spongiosus of the white matter similar to that seen in phenylketonuria and nonketotic hyperglycinemia [36]. The changes have generally been described in infants nine months to 4.5 years of age, but spongiform change was reported along with edema in an infant who died at 12 days of life [37]. Patients in the original series [2] who died at 11 and 14 days had cerebral edema. Spongiform changes and intramyelin vacuoles on electron microscopy have been observed in an animal model in which Hereford calves died of MSUD within the first week of life [38].

A number of variants of branched-chain oxoaciduria has now been described in addition to the classic, severe, or neonatal form [39]. Each is milder in its clinical presentation than classic MSUD. The first of these to be described [40–44] has been referred to as intermittent branched-chain ketoaciduria. Involved individuals may have no problems except in the presence of some special stress, such as infection or surgery. On the other hand,

this disorder too can be lethal. Patients with no symptoms at all for a period of years can suddenly develop coma, convulsions, and death following an apparently mild infection [44]. More commonly, these patients have intermittent bouts of acute ataxia. In one report, severe acidosis was a prominent clinical finding [43].

A third form has been referred to as intermediate branched-chain ketoaciduria [45–47]. These patients usually presented with mental impairment and hence some symptomatology was considered to be continuous as opposed to intermittent. In previous editions of this book, we have cited our experience that the biochemical abnormality of the accumulation of amino acids and oxoacids in body fluids is not intermittent, even though the clinical manifestations may be. In all of these patients, the biochemical features were always demonstrable, except of course when successfully treated. We have now encountered patients who clearly fit the definition of intermittent even of the biochemistry. Nevertheless, it is increasingly clear that what we are dealing with is a continuum.

One patient reported as intermediate [47] presented first with ketoacidosis and coma at the age of ten months and subsequently responded to dietary therapy with no further episodes and had an IQ of 92. Others described as intermittent have had levels of activity indistinguishable from those with classic phenotypes [48, 49]. Some have presented with ophthalmoplegia [50, 51].

Dancis [39] based a classification of variants on protein intolerance. In the classic form of the disease, he considered the patients unable to tolerate maintenance requirements of protein and requiring artificial purified amino acid diets for survival. Enzyme levels were 0–2 percent of normal. In the second group, protein tolerance was sufficient to maintain normal growth in infancy or 1.5–2.0 g/kg of protein. Enzyme levels in this group were between 2 and 8 percent of normal. In the third group, an unrestricted diet was tolerated. Enzyme activity was between 8 and 16 percent of normal.

Oxidation of labeled leucine *in vivo* served as a better discriminant than *in vitro* enzyme activity of variant forms of MSUD [52]. In patients with classical infantile disease, it was unmeasurable. In severe variants, it was 4 percent of control. In six milder variants, it ranged from 19 to 86 percent of control.

Another variant may be distinguished by the fact that the biochemical abnormalities are corrected by the administration of high doses of thiamine [53]. This thiamine-responsive MSUD or thiamine-responsive branched-chain oxoaciduria was originally described in a patient with relatively mild clinical symptomatology who responded to as little as 10 mg of thiamine per day [53]. Patients described to date have all had residual activity of the enzyme [54, 55], but doses up to 300 mg/day have been required, and two of them presented with classical clinical disease in infancy [56]. These patients have been quite heterogeneous and nutritional therapy has been necessary.

A patient with E3 deficiency [56] presented with feeding

difficulties in the first week, vomiting, and failure to thrive. By 6 weeks, severe developmental delay was apparent along with hypotonia and very poor head control. At eight months, he had lactic acidosis (10 mmol/L) and respiratory distress. He died in severe acidosis following liver biopsy at 18 months of age. E3 deficiency is considered in [Chapter 50](#).

GENETICS AND PATHOGENESIS

MSUD is transmitted as an autosomal recessive trait. This is true of each of the variants. Classic MSUD has been seen throughout the world and in all ethnic groups. It is common among the Mennonites of Pennsylvania in whom the incidence is one in 760. In the New England screening program, an incidence of one in 290,000 was encountered [57]. Heterozygote detection by enzymatic assay may not be reliable. Once a mutation is identified in a proband, molecular techniques may be used to establish carrier status. The activity of the enzyme can be measured in cultured amniotic fluid cells, and the disease has been diagnosed prenatally in a substantial number of patients. Mutations can readily be tested for in prenatal diagnosis, especially with allele-specific oligonucleotide probes [12, 58].

The molecular defect in MSUD is in the branched-chain oxoacid dehydrogenase which catalyzes the decarboxylation of the oxoacids ([Figures 19.1 and 19.2](#)). Activity is widely distributed in mammalian tissues. The enzyme is located on the inner surface of the inner mitochondrial membrane. Activity can be measured in human liver, kidney, and leukocytes, cultured fibroblasts or lymphoblasts, and amniotic fluid cells [59–62].

In the reaction, as in case of pyruvate or α -ketoglutarate, there is first a thiamine pyrophosphate (TPP)-mediated conversion of the carboxyl group to CO_2 and the formation of a covalently bound enzyme, TPP, substrate complex ([Figure 19.2](#)). Next, there is an oxidative transfer to the second, lipoic acid-bearing enzyme, liberating TPP after which there is transfer to coenzyme A, and lipoic acid is regenerated. Regulation of enzyme involves acylCoA compounds, and activity is stimulated by carnitine [63], presumably by the formation of acylcarnitine esters of acylCoA compounds and prevention of product inhibition. The enzyme is inhibited by adenosinediphosphate (ADP), a condition under which pyruvate decarboxylation is stimulated. Additional regulation has been demonstrated in a phosphorylation/dephosphorylation cycle in which the dehydrogenase complex is inactivated by a kinase catalyzed phosphorylation and activated through action of the phosphatase.

Measurement of the activity of branched-chain ketoacid decarboxylase *in vitro* in fibroblasts or leukocytes has generally been carried out by studying the conversion of leucine- ^{14}C , isoleucine- ^{14}C , valine- ^{14}C , or α -oxoisocaproic acid- ^{14}C to $^{14}\text{CO}_2$. In patients with classic MSUD, each activity has been virtually nil [6, 64]. In contrast, the oxidation of isovaleric acid- ^{14}C to $^{14}\text{CO}_2$ is normal.

Patients with intermittent branched-chain oxoaciduria and other variants have been found to have residual activity [6, 39]. Activity of up to 15–25 percent of the normal level has been observed [47]. A patient with E3 deficiency had defective activity of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase, as well as BKAD [56].

Abnormal activities have been identified in the individual components of the enzyme complex [65], but these assays are demanding, and dissection of the individual components in patients has been facilitated by northern and western blotting with specific antibodies and cDNA probes [65, 66]. More recently, this dissection has been done by retroviral complementation of dehydrogenase activity using plasmids containing the wild type for each of the three genes E1 α , E1 β , and E2; the one that restored activity identified the mutated gene [67]. There may be functional deficiency of E1 β and lack of immunoreactive protein as a consequence of mutation in E1 α [12], consistent with a requirement for protein interactions in assembly and stabilization.

Mutational analysis has identified a number of the MSUD mutations in E1 α [68].

The vast majority of these have been missense mutations and most have been associated with the severe, classic phenotype. The most prevalent mutation, the T to A change found in Mennonites [12], has also been found in other populations [68]. Of three other missense mutations in E1 α , one led to a classical phenotype [68], and two were intermediate in Mexican-American patients [69]. The G245R mutation appears to be common in that population. Four E1 α mutations are common in Japanese [70].

A founder effect and mutational hot-spot mutation has been reported c.11/del C in Portuguese gypsies in whom MSUD is common [71]. A patient detected by newborn screening was found to be developing normally and passed rigorous psychometric assessment despite the presence of the characteristic biochemical phenotype, including alloisoleucine. He was found to have a novel intronic change c.288+9C>T in the *BCKDHA* gene on one allele and p.G249S, a mutation previously associated with severe phenotype on the other [72]. He was found to have variably spliced products, including some normal mRNA. In the E1 β gene, many mutations were found in Japanese [70]. An 11 base pair deletion in the mitochondrial target sequence is relatively common [73]. Five mutations were found [71] in Portuguese, three of them nonsense, p.P200X, Q267Y, and R285X.

A number of mutations have been found in E2 [67, 74–78]. They include single base substitutions [73–76], insertions [69, 75], and deletions [75, 77, 79], and these mutations have led to missense, nonsense, frame shift, and internal deletion, as well as exon skipping at splice junctions and coding regions. Many have been compounds of two mutations [74–77]. Three novel missense mutations and a frame shift were found in Portuguese patients [71]. The E2 gene appears to have a propensity for splicing errors, some induced by large mutations in introns. The original thiamine-responsive patient of Scriver *et al.* [53] had a 17 base pair insertion in the E2 mRNA [74], resulting from a deletion in intron 4 [78]. Many of the E2 variants have been seen in patients with clinical variant phenotypes.

Five missense mutations have been reported in E3 in a single compound patient [78, 80]. A G229C mutation is common in Ashkenazi Jews [80].

Defective activity of the dehydrogenase complex leads to elevated concentrations of leucine, isoleucine, and valine in the plasma and urine (Table 19.1) [3, 15, 16].

Patients also excrete the oxoacid products of the transamination of each of these amino acids (Table 19.2), consistent with the site of enzymatic block (Figure 19.1) [15, 16, 81]. Isovaleric acid, α -methylbutyric acid, and isobutyric acid are not found. Among the amino acids, the concentration of leucine is virtually always higher than those of isoleucine and valine [3]. An exception was a variant patient described as valine-toxic [82]. Two other patients were unusual in that most of the branched chain oxoacids were derived from isoleucine [83, 84]. The oxoacid analogs of leucine and isoleucine are usually present in much higher quantities than the corresponding hydroxyacids. In contrast, 2-hydroxyisovaleric acid is usually present in much higher concentration than its oxoacid. Alloisoleucine is also regularly found [81]. This product of isoleucine accumulation was originally mistaken for methionine in the amino acid analyzer, creating some confusion in the management of early patients. The concentration of alanine in the plasma of these patients

Table 19.1 Concentrations of amino acids in plasma in untreated maple syrup urine disease

Valine ($\mu\text{mol/L}$)	Isoleucine ($\mu\text{mol/L}$)	Leucine ($\mu\text{mol/L}$)	Alloisoleucine ($\mu\text{mol/L}$)
500–1800	200–1300	500–5000	Trace–300

Table 19.2 Organic acids of the urine in maple syrup urine disease

Oxo acids (mmol/m creatinine)			Hydroxy acids (mmol creatinine)		
2-Oxoisocaproic acid	2-Oxo-3-methylvaleric acid	2-Oxoisovaleric acid	2-Hydroxy-isocaproic acid	2-Hydroxy-3-methylvaleric acid	2-Hydroxy-isovaleric acid
400–4400	500–2500	600–800	3–8	60–400	850–3600

is decreased [14]. During illness as the leucine rises, the alanine falls. The molar ratio of leucine to alanine is a more sensitive measure than leucine alone, and may be useful in diagnosis or neonatal screening [20]. In a series of 18 newborns with MSUD, the ratio was 1.3 to 12.4 (normal, 0.12–0.53).

Screening for the disease has been carried out by the addition of 2,4-dinitrophenylhydrazine, which produces a yellow precipitate of dinitrophenylhydrazones [85] in the presence of oxoacids. The individual oxoacids have been distinguished by gas chromatography-mass spectrometry (GCMS) of the oximes [86]. The ferric chloride test on the urine may yield a greenish-gray color.

Rapid screening for MSUD is now done best by tandem mass spectrometry (MS/MS), and this forms the basis for all of the neonatal screening programs for this disease. The method is not useful for management because it does not distinguish between leucine and isoleucine, but this is not a problem for screening. The leucine (isoleucine) alanine ratio [20] should be a useful assessment in these programs. Actually, hydroxyproline and alloisoleucine are isobaric with leucine and isoleucine; so newborn screening does not separate any of them. A positive screen is followed up by plasma amino acid analysis, which distinguishes all of these and rules out hydroxyprolinemia. A liquid chromatography mass spectrometry (LC-MS/MS) method has been developed for alloisoleucine which serves as a second tier test for newborn screening of dried blood spots [87].

TREATMENT

Emergency treatment of an infant in coma requires prompt reduction of levels of leucine and the other branched-chain amino acids. This has formerly been approached by exchange transfusions, peritoneal dialysis, or both; but direct measurements have indicated the removal of small quantities of amino acids in this way. Hemodialysis is doubtless effective, but it is formidable in a young infant, and the prospect of repeat dialysis with each respiratory infection in the early years of life is impossible to consider. Recently, Saudubray and colleagues [88, 89] have reported on continuous venovenous extracorporeal hemodiafiltration as a more rapid method for the lowering of high levels of leucine. In six neonatal infants and six children with later episodes, this approach was begun after 6 hours of conservative management including enteral amino acid mixtures, and leucine concentration, was over 1700 $\mu\text{mol/L}$. In each, the decrease in leucine was logarithmic and usually reached <1000 $\mu\text{mol/L}$ in 24 hours, while the rate of decrease with enteral therapy after cessation of the diafiltration was a slower linear fall. Follow-up developmental levels in this series were encouraging; some had intelligence quotients over 100 with follow up of as long as three to five years.

The alternative approach has been to take advantage

of the power of the anabolic laying down of accumulated amino acids into protein to lower toxic levels of branched-chain amino acids. This can be accomplished by providing mixtures of amino acids not containing leucine, isoleucine, and valine and energy as provided by 10 percent glucose intravenously. Intravenous solutions have been developed for this purpose and shown to be rapidly effective [90–92], but these solutions are not generally available, and are very expensive. We have successfully employed 200 mL/kg of 10 percent glucose intravenously and 2 g/kg of amino acid mixture providing 88 cal/kg. Berry and colleagues [91] have used larger quantities of glucose, requiring a central line and insulin. In a patient who is not vomiting, it is also possible to accomplish this by intragastric drip. Even in the presence of vomiting, we have found that provision of enteral amino acid mixtures dripped in minimal volume over 24 hours are usually tolerated [93, 94]. Our mixtures contained extra quantities of alanine and glutamine [94] and so do those employed by Morton *et al.* [20]. In their extensive experience, the rate of fall of plasma leucine with this approach was consistently greater than those reported for dialysis or hemoperfusion [20]. Commercial mixtures suitable for enteral use in minimal volume for the management of the acute episodes are now available in the United States (Complex[®] MSUD Amino Acid Blend; Applied Nutrition, Randolph, NJ, USA). A protocol for acute management is given in Table 19.3. The table provides

Table 19.3 Management of the acute crisis in maple syrup urine disease

Stop oral feedings. Begin intravenous hydration with 10% glucose and maintenance electrolyte.

Begin nasogastric or gastrostomy drip with 2.0–4.0 g/kg of amino acid protein equivalent – lacking leucine, isoleucine, and valine.

Complex MSUD Amino Acid Blend: 13 g of mixture provides 10 g of amino acids – 2.0 g/kg = 2.6 g/kg of complex. This contains 8 cal/kg. Add H_2O to make 8 mL/kg or 1 cal/mL to make for minimal volume in a vomiting or potentially vomiting patient, and drip this volume in slowly over 24 hours.

Obtain plasma stat for amino acids at least every 12 hours initially until therapeutic trend established; thereafter at least daily.

Plan to add isoleucine even in the first 24 hours, as hypoisoleucinemia will stop anabolism, lead to catabolism and consequent rise in leucine. A level of 20 mg/kg of added isoleucine is usually sufficient, but if added later even 100 mg/kg may be required. Valine supplementation may also be necessary before a steady-state leucine level is achieved.

In patients needing additional therapy add insulin (0.1 U/kg per hour). Provide intravenous glucose as 10% – at least 20 mL/U insulin. Monitor blood sugar and urine – dipstick and adjust.

In patients needing additional therapy, add s.c. human growth hormone 0.05 mg/kg per 24 hours.

Thiamine at 100 mg/kg can be given parenterally at the start of therapy. Patient could be discharged with p.o. allithiamine and later tested to see if thiamine added anything to treatment.



Figure 19.11 A six-month-old Saudi patient with maple syrup urine disease, who developed isoleucine/valine deficiency and its skin manifestations that often complicate the treatment of this disease. Cutaneous manifestations receded after supplementation with isoleucine 10 mg/kg per day and valine 60 mg/kg per day.

an approach to the prevention of deficiencies of isoleucine and/or valine that regularly occurs during leucine reduction, because there is virtually always more leucine than the other branched-chain amino acids. Deficiency of either of these essential amino acids leads to breakdown of the skin (Figure 19.11).

Wendel and colleagues [95] treated the acute crisis with insulin and glucose as an anabolic approach to therapy. In each episode studied, the introduction of this regime led to reduction in toxic levels of leucine. Studies of the *in vivo* metabolism of ^{13}C -leucine have indicated that protein synthesis is normal, and that there is no significant route for disposal of leucine other than protein synthesis [96], providing further argument for anabolic approaches to therapy. In these studies, leucine oxidation was undetectable, consistent with what we have found in fibroblasts *in vitro* [97]. We have recently employed enteral anabolic therapy, parenteral insulin, and glucose along with human growth hormone (Table 19.3).

Chronic management consists of restricting the intake of each of the three branched-chain amino acids to those essential for growth and no more. This type of dietary management is much more difficult than that of phenylketonuria. It requires very close regulation of an artificial diet and frequent access to an amino acid analyzer. The best results are seen in those in whom treatment has been initiated earliest. The largest experience with the management of this disease is that of Snyderman *et al.* [14, 98] and Morton *et al.* [20], and both have written that there

can be little doubt about the beneficial effect of therapy in this disorder. Commercial products are available that are useful in the management of this disorder (Ketonex-Ross, MSUD-Mead-Johnson) [99].

In thiamine-responsive MSUD, the doses employed have ranged from 10 to 300 mg/day [53, 55]. It has appeared reasonable to test each patient with larger amounts before deciding that thiamine is not a useful adjunct to therapy. However, this is complicated by data that indicate not more than a few milligrams of an oral dose are absorbed [100], implying that parenteral administration may be necessary to assess the effects of larger doses. Allithiamine may be useful orally.

The management of intercurrent illness is particularly important in this disease [101], and parents must be taught to be efficient partners in recognition and prompt management. Written protocols or letters to Emergency Room physicians for use when illness occurs in out of town situations are useful adjuncts. A regimen of restriction of leucine intake at the first sign of illness, continuation of other amino acids (including 10 mg/kg of isoleucine and of valine) and the supply of abundant calories particularly as glucose or glucose polymer is useful.

Liver transplantation is an option in the treatment of MSUD [102–106]. Orthotopic liver transplantation was carried out in two patients with the disease for nonmetabolic reasons. Both had liver failure, one from hepatitis A [102] and the other from intoxication with vitamin A [103]. Both have remained metabolically and neurologically stable without any restriction of protein intake for over two years. A third patient [5, 104] was transplanted because of the request of parents concerned with delayed psychomotor development and frequent metabolic decompensation. In each patient, dramatic decrease in plasma levels of branched-chain amino acids occurred immediately, reaching near normal levels in 10 hours despite post-transplant catabolic stress. None of the patients had a further episode of metabolic derangement. Whole-body stable isotope-labeled leucine oxidation was normal in the one patient tested. Another patient has received a liver transplant because of liver failure [106]. At nine years, neurological findings included stupor, dystonia, ataxia, hyperreflexion, and positive Babinski signs. Five years following the procedure, neurological examination was normal as were plasma amino acid concentrations and calculated brain uptake of neutral amino acids.

Domino hepatic transplantation was reported [107, 108] in a 25-year-old man with MSUD who had had a number of recent admissions to hospital for metabolic imbalance. He had none post-transplantation. His liver was given to a 53-year-old man with cancer who otherwise would not have qualified for a liver. Both patients remained metabolically stable while receiving completely normal intakes of protein. Studies of ^{13}C -leucine oxidation to expired $^{13}\text{CO}_2$ revealed both patients to have levels intermediate between their pretransplant levels. Neither had appreciable alloisoleucine.

A major experience with hepatic transplantation

in MSUD was recently published [109] representing a collaboration between the Clinic for Special Children, Strasburg, PA where MSUD is common and the transplant center at the University of Pittsburgh. Eleven patients were reported. All were alive and well at report after 106 months in the index patient and 4–18 months (median 14 months) in the subsequent ten. Leucine levels were stable on an unrestricted diet and patients remained stable during protein loading and intercurrent illness.

REFERENCES

1. Peinemann F, Danner DJ. Maple syrup urine disease: 1954 to 1993. *J Inherit Metab Dis* 1994; **17**: 3.
2. Menkes JH, Hurst PL, Craig JM. New syndrome: progressive infantile cerebral dysfunction associated with unusual urinary substance. *Pediatrics* 1954; **14**: 462.
3. Westall RG, Dancis J, Miller S. Maple syrup urine disease – a new molecular disease. *Am J Dis Child* 1957; **94**: 571.
4. Menkes JH. Maple syrup urine disease: isolation and identification of organic acids in the urine. *Pediatrics* 1959; **23**: 348.
5. Dancis J, Hutzler J, Levitz M. Metabolism of the white blood cells in maple-syrup-urine disease. *Biochim Biophys Acta* 1960; **43**: 342.
6. Dancis J, Hutzler H, Snyderman SE, Cox RP. Enzyme activity in classical and variant forms of maple syrup urine disease. *J Pediatr* 1972; **81**: 312.
7. Pettit FH, Yeaman SJ, Reed LJ. Purification and characterization of branched chain α -keto acid dehydrogenase complex of bovine kidney. *Proc Natl Acad Sci USA* 1978; **75**: 4881.
8. Wynn RM, Song J, Chuang DT. GroEL/GroES promote dissociation/reassociation cycles of a heterodimeric intermediate during $\alpha 2 \beta 2$ protein assembly. *J Biol Chem* 2000; **275**: 2786.
9. Fekete J, Plattner R, Crabb DW *et al.* Localization of the human gene for the E1 α subunit of branched chain keto acid dehydrogenase (BCKDHA) to chromosome 19q131–q132. *Cytogenet Cell Genet* 1989; **50**: 236.
10. Zneimer SM, Lau KS, Eddy RL *et al.* Regional assignment of two genes of the human branched-chain α -keto acid dehydrogenase complex: the E1 β gene (BCKDHB) to chromosome 6p21–22 and the E2 gene (DBT) to chromosome 1p3. *Genomics* 1991; **10**: 740.
11. Scherer SW, Otulakowski G, Robinson BH, Tsui LC. Localization of the human dihydrolipoamide dehydrogenase gene (DLD) to 7q31–q32. *Cytogenet Cell Genet* 1991; **56**: 176.
12. Matsuda I, Nobukuni Y, Mitsubuchi H *et al.* A T-to-A substitution in the E1 α subunit gene of the branched-chain α -ketoacid dehydrogenase complex in two cell lines derived from Mennonite maple syrup urine disease patients. *Biochem Biophys Res Commun* 1990; **172**: 646.
13. Edelman L, Wasserstein MP, Diaz GA *et al.* Maple syrup urine disease: identification and carrier-frequency determination of a novel founder mutation in the Ashkenazi Jewish population. *Am J Hum Genet* 2001; **69**: 863.
14. Snyderman SE. Maple syrup urine disease. In: Nyhan WL (ed.). *Heritable Disorders of Amino Acid Metabolism*. New York: J Wiley & Sons, 1960: 17–31.
15. Dancis J, Levitz M, Westall RG. Maple syrup urine disease: branched chain keto-aciduria. *Pediatrics* 1960; **25**: 72.
16. Mackenzie DY, Woolf LI. Maple syrup urine disease: inborn error of metabolism of valine leucine and isoleucine associated with gross mental deficiency. *Br Med J* 1959; **1**: 90.
17. Mantovani JF, Naidich TP, Prensky AL *et al.* MSUD: presentation with pseudotumor cerebri and CT abnormalities. *J Pediatr* 1980; **96**: 279.
18. Riviello JJ Jr, Rezvani I, DiGeorge AM, Foley CM. Cerebral edema causing death in children with metabolic disease. *J Pediatr* 1991; **119**: 42.
19. Mikati MA, Dudin GE, Der Kaloustian VM *et al.* Maple syrup urine disease with increased intracranial pressure. *Am J Dis Child* 1982; **136**: 642.
20. Morton DH, Strauss KA, Robinson DL *et al.* Diagnosis and treatment of maple syrup disease: a study of 36 patients. *Pediatrics* 2002; **109**: 999.
21. Hauser GJ, Chitayat D, Berns L *et al.* Peculiar odours in newborns and maternal prenatal ingestion of spicy food. *Eur J Pediatr* 1985; **144**: 403.
22. Bartley GB, Hilty MD, Anderson BD *et al.* 'Maple syrup' urine odor due to fenugreek ingestion. *N Engl J Med* 1981; **305**: 467.
23. Monastiri K, Limame D, Kaabachi N *et al.* Fenugreek odour in maple syrup urine disease. *J Inherit Metab Dis* 1997; **20**: 614.
24. Podebrad F, Heil M, Reichert S *et al.* 45-Dimethyl-3-hydroxy-2[5H]-furanone (sotolone) – the odour of maple syrup urine disease. *J Inherit Metab Dis* 1999; **22**: 107.
25. Donnell GN, Lieberman E, Shaw KNF, Koch R. Hypoglycemia in maple syrup urine disease. *Am J Dis Child* 1967; **113**: 60.
26. Treacy E, Clow CL, Reade TR *et al.* Maple syrup urine disease: interrelations between branched-chain amino- oxo- and hydroxyacids; implications for treatment associations with CNS demyelination. *J Inherit Metab Dis* 1992; **15**: 121.
27. Kahler SG, Sherwood GW, Woolf D *et al.* Pancreatitis in patients with organic acidemias. *J Pediatr* 1994; **124**: 239.
28. Estivill E, Sanmarti FX, Vidal R *et al.* (Comb-like rhythm: an EEG pattern peculiar to leucinoses.) *An Esp Pediatr* 1985; **22**: 123.
29. Tharp BR. Unique EEG pattern (comb-like rhythm) in neonatal maple syrup urine disease. *Pediatr Neurol* 1992; **8**: 65.
30. Verdu A, Lopez Herce J, Pascual Castroviejo I *et al.* Maple syrup urine disease variant form: presentation with psychomotor retardation and CT scan abnormalities. *Acta Paediatr Scand* 1985; **74**: 815.
31. Snyderman SE. Treatment outcome of maple syrup urine disease. *Acta Paediatr Jpn* 1988; **30**: 417.
32. Suzuki S, Naito H, Abe T, Nihei K. Cranial computed tomography in a patient with a variant form of maple syrup urine disease. *Neuropediatrics* 1983; **14**: 102.
33. Romero FJ, Ibarra B, Rovira M *et al.* Cerebral computed tomography in maple syrup urine disease. *J Comput Assist Tomogr* 1984; **8**: 410.
34. Brismar J, Aqeel A, Brismar G *et al.* Maple syrup urine disease: findings on CT and MR scans of the brain in 10 infants. *Am J Neuroradiol* 1990; **11**: 1219.

35. Taccone A, Schiaffino MC, Cerone R *et al.* Computed tomography in maple syrup urine disease. *Eur J Radiol* 1992; **14**: 207.
36. Crome L, Dutton G, Ross CF. Maple syrup urine disease. *J Path Bact* 1961; **81**: 379.
37. Menkes JH, Philippart M, Fiol RE. Cerebral lipids in maple syrup urine disease. *J Pediatr* 1965; **66**: 584.
38. Harper PA, Healy PJ, Dennis JA. Maple syrup urine disease as a cause of spongiform encephalopathy in calves. *Vet Rec* 1986; **119**: 62.
39. Dancis J. Variants of maple syrup urine disease. In: Nyhan WL (ed.). *Heritable Disorders of Amino Acid Metabolism*. New York: J Wiley & Sons, 1974: 32–6.
40. Dancis J, Hutzler J, Rokkones T. Intermittent branched-chain ketonuria. Variant maple-syrup-urine disease. *N Eng J Med* 1967; **276**: 84.
41. Morris MD, Lewis BD, Doolan PD, Harper HA. Clinical and biochemical observations on an apparently nonfatal variant of branched-chain ketoaciduria. *Pediatrics* 1961; **28**: 918.
42. Kiil R, Rokkones T. Late manifesting variant of branched-chain ketoaciduria. *Acta Paediatr Scand* 1964; **53**: 356.
43. Van Der Hort HL, Wadman SK. A variant form of branched-chain ketoaciduria. *Acta Paediatr Scand* 1971; **60**: 594.
44. Goedde HW, Langenbeck V, Brackertz D *et al.* Clinical and biochemical genetic aspects of intermittent branched-chain ketoaciduria. *Acta Paediatr Scand* 1967; **59**: 83.
45. Schulman JD, Lustberg TJ, Kennedy JL *et al.* A new variant of maple syrup urine disease (branched-chain ketoaciduria). *Am J Med* 1970; **49**: 118.
46. Fischer MH, Gerritsen T. Biochemical studies on a variant of branched-chain ketoaciduria in a nineteen-year-old female. *Pediatrics* 1971; **48**: 795.
47. Gonzalez Rios MC, Chuang DT, Cox RP *et al.* A distinct variant of intermediate maple syrup urine disease. *Clin Genet* 1985; **27**: 153.
48. Valman HB, Patrick AD, Seakins JW *et al.* Family with intermittent maple syrup urine disease. *Arch Dis Child* 1973; **48**: 225.
49. Dent CE, Westall RG. Studies in maple syrup urine disease. *Arch Dis Child* 1961; **36**: 259.
50. Chhabria S, Tomasi LG, Wong PW. Ophthalmoplegia and bulbar palsy in variant form of maple syrup urine disease. *Ann Neurol* 1979; **6**: 71.
51. MacDonald JT, Sher PK. Ophthalmoplegia as a sign of metabolic disease in the newborn. *Neurology* 1977; **27**: 971.
52. Schadowaldt P, Bodner-Leidecker A, Hammen H-W, Wendel U. Whole-body L-leucine oxidation in patients with variant form of maple syrup urine disease. *Pediatr Res* 2001; **49**: 627.
53. Scriver CR, Clow CL, Mackenzie S, Delvin E. Thiamine-responsive maple syrup urine disease. *Lancet* 1971; **1**: 310.
54. Duran M, Wadman SK. Thiamine-responsive inborn errors of metabolism. *J Inherit Metab Dis* 1985; **8**: 70.
55. Fernhoff PM, Lubitz D, Danner DJ *et al.* Thiamine response in maple syrup urine disease. *Pediatr Res* 1985; **19**: 1011.
56. Munnich A, Saudubray JM, Taylor J *et al.* Congenital lactic acidosis alpha-ketoglutaric aciduria and variant form of maple syrup urine disease due to a single enzyme defect: dihydrolipoyl dehydrogenase deficiency. *Acta Paediatr Scand* 1982; **71**: 167.
57. Naylor EW. Newborn screening for maple syrup urine disease. In: Therrell BL (ed.). *Laboratory Methods for Neonatal Screening*. Washington DC: American Public Health Association, 1993: 115–24.
58. Zhang B, Edenberg HJ, Crabb DW, Harris RA. Evidence for both a regulatory mutation and a structural mutation in a family with maple syrup urine disease. *J Clin Invest* 1989; **83**: 1425.
59. Dancis J, Hutzler J, Levitz M. Tissue distribution of branched chain keto-acid decarboxylase. *Biochim Biophys Acta* 1961; **52**: 60.
60. Dancis J, Jansen V, Hutzler J, Levitz M. The metabolism of leucine in tissue culture of skin fibroblasts of maple syrup urine disease. *Biochim Biophys Acta* 1963; **77**: 523.
61. Elsas LJ, Priest JH, Wheeler FB *et al.* Maple syrup urine disease: coenzyme function and prenatal monitoring. *Metabolism* 1974; **23**: 569.
62. Wendel U, Rudiger HW, Passarge E, Mikkelsen M. Maple syrup urine disease: rapid prenatal diagnosis by enzyme assay. *Humangenetik* 1973; **19**: 127.
63. Aftring RP, May ME, Buse MG. Regulation of branched chain ketoacid metabolism in rat liver. In: Walser M, Williamson JR (ed.). *Metabolism and Clinical Implications of Branched Chain Amino and Ketoacids*. Amsterdam: Elsevier North Holland, 1981: 67–72.
64. Dancis J, Hutzler J, Levitz M. The diagnosis of maple syrup urine disease (branched-chain ketoaciduria) by the *in vitro* study of the peripheral leukocyte. *Pediatrics* 1963; **32**: 234.
65. Indo Y, Akaboshi I, Nobukuni Y *et al.* Maple syrup urine disease: a possible biochemical basis for clinical heterogeneity. *Hum Genet* 1988; **80**: 6.
66. Nobukuni Y, Mitsubuchi H, Ohta K *et al.* Molecular diagnosis of maple syrup urine disease: screening and identification of gene mutations in the branched-chain α -ketoacid dehydrogenase multienzyme complex. *J Inherit Metab Dis* 1992; **15**: 827.
67. Nellis MN, Danner DJ. Gene preference in maple syrup urine disease. *Am J Hum Genet* 2001; **68**: 232.
68. Chuang JL, Fisher CR, Cox RP, Chuang DT. Molecular basis of maple syrup urine disease: novel mutations at the E1 α locus that impair E1 (α 2 β 2) assembly or decrease steady-state E1 α mRNA levels of branched-chain α -keto acid dehydrogenase complex. *Am J Hum Genet* 1994; **55**: 297.
69. Chuang JL, Davie JR, Chinsky JM *et al.* Molecular and biochemical basis of intermediate maple syrup urine disease. Occurrence of homozygous G245R and F364 C mutations at the E1 α locus of Hispanic-Mexican patients. *J Clin Invest* 1995; **95**: 954.
70. Nobukuni Y, Mitsubuchi H, Hayashida Y *et al.* Heterogeneity of mutations in maple syrup urine disease (MSUD): screening and identification of affection E1 α and E1 β subunits of the branched-chain α -keto-acid dehydrogenase multienzyme complex. *Biochim Biophys Acta* 1993; **1225**: 64.
71. Quental S, Gusmão A, Rodríguez-Pombo P *et al.* Revisiting MSUD in Portuguese gypsies: evidence for a founder mutation

- and for a mutational hotspot within the BCKDHA gene. *Ann Hum Genet* 2009; **73**: 298.
72. Fernández-Guerra P, Navarrete R, Weisiger K *et al*. Functional characterization of the novel intronic nucleotide change c.288+9C>T within the BCKDHA gene: understanding a variant presentation of maple syrup urine disease. *J Inherit Metab Dis* 2010; April 30 [Epub ahead of print].
 73. Nobukuni Y, Mitsubuchi H, Akaboshi I *et al*. Maple syrup urine disease: complete defect of the E1-beta subunit of the branched chain alpha-ketoacid dehydrogenase complex due to a deletion of an 11-bp repeat sequence which encodes a mitochondrial targeting leader peptide in a family with the disease. *J Clin Invest* 1991; **87**: 1862.
 74. Fisher CW, Lau KS, Fisher CR *et al*. A 17-bp insertion and a Phe215-Cys missense mutation in the dihydrolipoyl transacylase (E2) mRNA from a thiamine-responsive maple syrup urine disease patient WG-34. *Biochem Biophys Res Commun* 1991; **174**: 804.
 75. Chuang DT, Fisher CW, Lau KS *et al*. Maple syrup urine disease: domain structure mutations and exon skipping in the dihydrolipoyl transacylase (E2) component of the branched-chain alpha-ketoacid dehydrogenase complex. *Mol Biol Med* 1991; **8**: 49.
 76. Fisher CW, Fisher CR, Chuang JL *et al*. Occurrence of a 2-bp (AT) deletion allele and a nonsense (G-to-T) mutant allele at the E2 (DBT) locus of six patients with maple syrup urine disease: multiple exon skipping as a secondary effect of the mutations. *Am J Hum Genet* 1993; **52**: 414.
 77. Herring WJ, McKean M, Dracopoli N, Danner DJ. Branched chain acyltransferase absence due to an Alu-based genomic deletion allele and an exon skipping allele in a compound heterozygote proband expressing maple syrup urine disease. *Biochim Biophys Acta* 1992; **1138**: 236.
 78. Chuang DT, Shih VE. Maple syrup urine disease (branched-chain ketoaciduria). In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic and Molecular Basis of Inherited Disease*, 8th edn. New York: McGraw Hill, 2001: 1971.
 79. Mitsubuchi H, Nobukuni Y, Akaboshi I *et al*. Maple syrup urine disease caused by a partial deletion in the inner E2 core domain of the branched chain alpha-keto acid dehydrogenase complex due to aberrant splicing. A single base deletion at a 59-splice donor site of an intron of the E2 gene disrupts the consensus sequence in this region. *J Clin Invest* 1991; **87**: 1207.
 80. Liu TC, Kim H, Arizmendi C *et al*. Identification of two missense mutations in a dihydrolipoamide dehydrogenase-deficient patient. *Proc Natl Acad Sci USA* 1993; **90**: 235.
 81. Norton PM, Roitman E, Snyderman SE, Holt Jr LE. A new finding in maple syrup urine disease. *Lancet* 1964; **1**: 26.
 82. Zipf WB, Hieber VC, Allen RJ. Valine-toxic intermittent maple syrup urine disease: a previously unrecognized variant. *Pediatrics* 1979; **63**: 286.
 83. Fischer MH, Gerritsen T. Biochemical studies on a variant of branched chain ketoaciduria in a 19-year-old female. *Pediatrics* 1971; **48**: 795.
 84. Gretter TE, Lonsdale D, Mercer RD *et al*. Maple syrup urine disease variant. Report of a case. *Cleve Clin Q* 1972; **39**: 129.
 85. Borden M. Methodology: screening for metabolic diseases. In: Nyhan WL (ed.). *Abnormalities in Amino Acid Metabolism in Clinical Medicine*. Norwalk, CT: Appleton Century Crofts, 1984: 401.
 86. Hoffmann G, Aramaki S, Blum-Hoffmann E *et al*. Quantitative analysis for organic acids in biological samples: batch isolation followed by gas chromatographic-mass spectrometric analysis. *Clin Chem* 1989; **38**: 587.
 87. Oglesbee D, Sanders KA, Lacey JM *et al*. Second-tier test for quantification of alloisoleucine and branched-chain amino acids in dried blood spots to improve newborn screening for maple syrup urine disease (MSUD). *Clin Chem* 2008; **54**: 542.
 88. Saudubray J-M, Ogier H, Charpentier C *et al*. Neonatal management of organic acidurias: clinical update. *J Inherit Metab Dis* 1984; **7**: 2.
 89. Jouvet P, Jugie M, Saudubray J-M *et al*. Combined nutritional support and continuous extracorporeal removal therapy in the severe acute phase of maple syrup urine disease. *Intens Care Med* 2001; **27**: 1798.
 90. Nyhan WL, Rice-Asaro M, Acosta P. Advances in the treatment of amino acid and organic acid disorders. In: Desnick RJ (ed.). *Treatment of Genetic Diseases*. New York: Churchill Livingstone 1991: 45.
 91. Berry GT, Heidenreich R, Kaplan P *et al*. Branched-chain amino acid-free parenteral nutrition in the treatment of acute metabolic decompensation in patients with maple syrup urine disease. *N Engl J Med* 1991; **324**: 175.
 92. Townsend I, Kerr DS. Total parenteral nutrition therapy of toxic maple syrup urine disease. *Am J Clin Nutr* 1982; **36**: 359.
 93. Parini R, Sereni LP, Bagozzi DC *et al*. Nasogastric drip feeding as the only treatment of neonatal maple syrup urine disease. *Pediatrics* 1993; **92**: 280.
 94. Nyhan WL, Rice-Kelts M, Klein J. Treatment of the acute crisis in maple syrup urine disease. *Arch Pediatr Adolesc Med* 1998; **152**: 593.
 95. Wendel U, Langenbeck U, Lombeck I, Bremer JH. Maple syrup urine disease: therapeutic use of insulin in catabolic states. *Eur J Pediatr* 1982; **139**: 172.
 96. Thompson GN, Bresson JL, Pacy PJ *et al*. Protein and leucine metabolism in maple syrup urine disease. *Am J Physiol* 1990; **258**: E654.
 97. Yoshida I, Sweetman L, Nyhan WL. Metabolism of branched-chain amino acids in fibroblasts from patients with maple syrup urine disease and other abnormalities of branched-chain ketoacid dehydrogenase activity. *Pediatr Res* 1986; **20**: 169.
 98. Snyderman SE, Norton PM, Roitman E, Holt Jr LE. Maple syrup urine disease with particular reference to diet therapy. *Pediatrics* 1964; **34**: 454.
 99. Smith BA, Waisman HA. Leucine equivalency system in managing branched chain ketoaciduria. *J Am Diet Assoc* 1971; **59**: 342.
 100. Haas RH. Thiamin and the brain. *Ann Rev Nutr* 1988; **8**: 383.
 101. Dixon MA, Leonard JV. Intercurrent illness in inborn errors of intermediary metabolism. *Arch Dis Child* 1992; **67**: 1387.
 102. Wendel U, Saudubray JM, Bodner A, Schadewaldt P. Liver transplantation in maple syrup urine. *Eur J Pediatr* 1999; **158**: S60.

103. Merinero B, Perez-Cerda C, Sanz P *et al*. Liver transplantation (LT) in a Spanish MSUD patient. 32nd Annual Symposium Society for the Study of Inborn Errors of Metabolism, Edinburgh, 1994: 64 (Abstr.).
104. Netter JC, Cossariza G, Narcy C *et al*. Devenir a moyen terme de deux cas de leucinose: place de la transplantation hepatique dans le traitement. *Arch Pediatr* 1994; **1**: 730.
105. Kaplan P, Mazur AM, Smith R *et al*. Transplantation for maple syrup urine disease (MSUD) and methylmalonic acidopathy (MMA). *J Inherit Metab Dis* 1997; **20**(Suppl. 1): 37 (Abstr.).
106. Robinson DL, Strauss KA, Puffenberger EG, Morton DH. Effects of liver transplant and bone marrow transplant upon amino acid homeostasis and the neuropathology of maple syrup urine disease. *Am J Hum Genet* 2002; **71**: 412.
107. Barshop BA, Khanna A. Domino hepatic transplantation in maple syrup urine disease. *N Engl J Med* 2005; **353**: 2410.
108. Khanna A, Hart M, Nyhan WL. Domino liver transplantation in maple syrup urine disease. *Liver Transplant* 2006; **12**: 876.
109. Strauss KA, Mazariegos GV, Sindhi R *et al*. Elective liver transplantation for the treatment of classical maple syrup urine disease. *Am J Transplant* 2006; **3**: 557.

Oculocutaneous tyrosinemia/tyrosine aminotransferase deficiency

Introduction	164	Treatment	168
Clinical abnormalities	165	References	169
Genetics and pathogenesis	167		

MAJOR PHENOTYPIC EXPRESSION

Dendritic keratitis, causing lacrimation, photophobia, inflammation, ulcers, and scars; keratoses of the palms and soles; hypertyrosinemia; defective activity of hepatic cytoplasmic tyrosine aminotransferase.

INTRODUCTION

Oculocutaneous tyrosinemia was first described in 1967 by Campbell and colleagues [1] in a report of a patient with corneal ulcers, erythematous papular lesions on the palms and soles, and severe impairment of mental development. A number of patients has since been reported, and it is clear that impaired mental development is not a uniform feature of the disease [2–8]. Patients were described in 1938 by Richner [9] and in 1947 by Hanhart [10] with typical lesions of the eyes and skin, and this came to be known

as the Richner–Hanhart syndrome, or keratosis palmaris et plantaris. It appears likely that oculocutaneous tyrosinemia and the Richner–Hanhart syndrome are the same disease, although plasma concentrations of tyrosine are not available for the original patients of Richner and Hanhart. Among the disorders in which elevated concentrations of tyrosine have been reported, this disorder appears to be a true hypertyrosinemia or tyrosine intoxication in the sense that the clinical manifestations are a consequence of the elevated levels of tyrosine. It has been referred to as ‘tyrosinemia type II’.

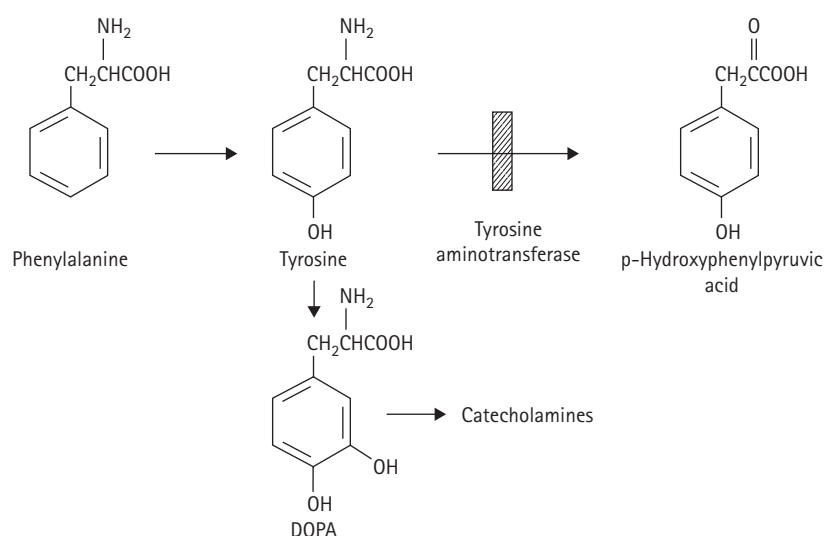


Figure 20.1 Metabolic pathways for tyrosine and the site of the defect in oculocutaneous tyrosinemia at the tyrosine aminotransferase step.

The enzymatic site of the defect in oculocutaneous tyrosinemia is in the hepatic tyrosine aminotransferase (TAT, L-tyrosine-2-oxoglutarate aminotransferase, EC 2.6.1.5) (Figure 20.1). There are two separate tyrosine aminotransferases, one in the cytosol and the other in the mitochondria. In this disorder, it is the cytosolic enzyme that is deficient [11–15]. The activity of the mitochondrial enzyme is normal. The gene for TAT is located on chromosome 16 at q22.1–22.3 [16, 17]. The gene has been cloned and sequenced [18]. A number of mutations has been defined [17, 18].

CLINICAL ABNORMALITIES

The most important manifestations of oculocutaneous tyrosinemia are those involving the eye [1, 2, 19], because they can lead to scarring of the cornea and permanent visual impairment. Ocular manifestations, such as lacrimation, photophobia, pain in the eye, or a history of red eyes are usually the initial manifestations of the disease and continue to be the most regularly encountered (Figure 20.2). Ocular symptoms may begin as early as the first day of life [20] and usually within the first years, but onset can be as late as 38 years [21], and documented patients have been asymptomatic [21]. Many patients have had symptoms of the eyes without cutaneous manifestations [5, 22–24], but others [6, 8, 24] had the reverse situation. Corneal ulcers are dendritic (Figures 20.3 and 20.4). The keratitis may resemble the dendritic keratitis of herpes.



Figure 20.2 KP: A nine-year-old girl with oculocutaneous tyrosinemia [2]. She had recurrent photophobia and conjunctival reddening from at least six months of age. Development was mildly impaired.

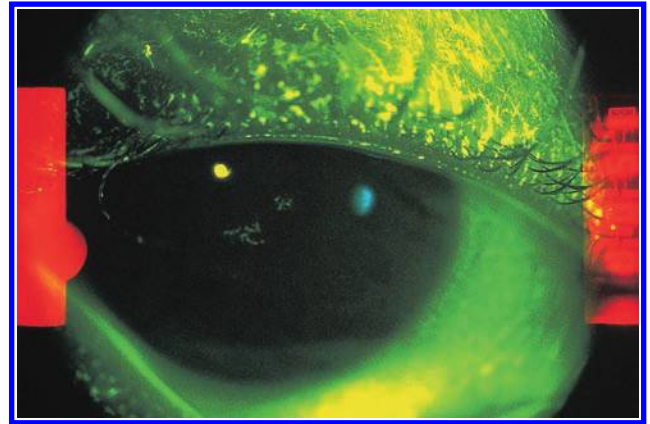


Figure 20.3 Corneal lesions of KP at nine years of age. The small dendritic lesion stained weakly with fluorescein. The lesion was slightly elevated. There was no ocular inflammation at the time. (Photograph kindly provided by Dr Perry Binder, San Diego, CA, USA.)

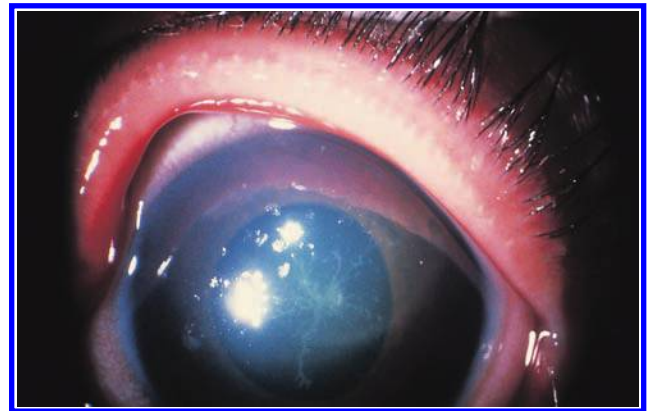


Figure 20.4 Corneal lesions in JF, a three-month-old patient with oculocutaneous tyrosinemia. The appearance was dendritic, but the lesions were elevated, opaque, and had mucoid material on the surface. The underlying dendritic figure stained weakly with fluorescein. (Illustration kindly provided by the US Naval Hospital, San Diego, CA, USA.)

The diagnosis may suggest itself to the ophthalmologist after cultures are negative or after a negative response to antiviral chemotherapy [2, 7]. Corneal erosions, ulcers, or plaques may be complications [25]. Ulcers may stain poorly with fluorescein. These lesions may lead to corneal clouding with central or paracentral opacities, scarring, and impaired vision. There may be neovascularization of the cornea. Keratitic lesions have occurred in a transplanted cornea [26]. A white film may be visible over the cornea. Glaucoma is another reported complication [27]. The differential diagnosis of keratitis in infancy or childhood is essentially herpes or this disease. Idiopathic keratitis does not occur in childhood. Therefore, the concentration of tyrosine in the blood should be determined in any pediatric patient with keratitis.

The ocular and the cutaneous lesions in this disease are the result of the accumulation of tyrosine. Intense burning of the eyes, hands, and feet have been observed within an hour of the administration of 0.7–0.8 mmol of tyrosine per kilogram [28], and erythema and pain have been observed in cutaneous lesions after a load of tyrosine [29]. Rats given a diet high in tyrosine develop keratitic ocular lesions [30]. Ocular abnormalities have been observed in other disorders in which tyrosine accumulates [31, 32].

The cutaneous lesions are painful keratoses which occur particularly on the peripheral pressure-bearing areas



Figure 20.5 The lesion on the left great toe of KP was thickened, scaly, and cracked. Plasma concentration of tyrosine was 912 mmol/L (16.5 mg/dL).



Figure 20.6 The foot of a Saudi patient with oculocutaneous tyrosinemia [34] who presented with these painful hyperkeratotic plaques.



Figure 20.7 The foot of the same patient 6 weeks after dietary restriction lowered blood concentrations of tyrosine.

of the palms and soles (Figures 20.5, 20.6, and 20.7) [33, 34]. They may occur near the tips of the digits. Subungual lesions may be found. Typical hyperkeratotic lesions are papular, well-demarcated plaques with irregular borders. Diameters up to 2 cm are common, but lesions may be larger and may be hollow or eroded, progressing to crusted, hyperkeratotic areas. They are not pruritic. They are painful and may be associated with hyperhydrosis [35]. They may be heralded by the appearance of blisters. Pain may be so severe that the patient will not walk. Skin lesions may be seen early in life or as late as the second decade [24]. Grayish hyperkeratotic plaques may be seen on the knees, elbows, or ankles. Hyperkeratoses have also been reported on the tongue [36]. Skin biopsy may reveal acanthosis and parakeratosis, as well as hyperkeratosis [33], none of them very specific findings. Electron microscopy may reveal unique thickening of the granular layer and increased tonofibrils and keratohyalin in the palmar and plantar skin along with large numbers of microtubules and unusually tight packing [37]. Crystals of tyrosine were not seen, but they have been seen in the cornea.

Neurologic features of the disease are quite variable [2, 24]. About half of the patients reported have had impaired mental development, a few severely so [11, 26, 38], but in most of them the level of intelligence was not very low [6, 8, 33, 36, 39]. Some have been described as having low–normal intelligence [8, 27, 28, 40, 41]. Two patients had normal intelligence, but had a learning disability [2, 38]. Among the patients described, there was no obvious relationship between the levels of intelligence and the levels of tyrosine

in the blood [2], and it is not clear whether impairment of mental development is an integral component of the syndrome or a reflection of ascertainment bias and the frequency with which individuals are studied for the possible occurrence of metabolic disease.

Hyperactivity has been observed in a number of patients, as well as abnormal language development [19]. An infant may be irritable. The first patient described [1] had self-injurious behavior, but had severely impaired mental development; this type of behavior has not been seen in others. Convulsions and microcephaly have been reported [42].

Maternal tyrosinemia has been documented to have adverse effects on the fetus [43]. An untreated 28-year-old woman whose disease was untreated had two pregnancies with tyrosine levels at around 1302 $\mu\text{mol/L}$. Both infants had microcephaly and delayed development. One had maxillary hypoplasia. Two other infants of untreated women had microphthalmia and impaired mental development [42].

GENETICS AND PATHOGENESIS

Transmission of oculocutaneous tyrosinemia is autosomal recessive. Consanguinity has been documented [4, 6, 33, 38, 39], as has the occurrence of more than one involved sibling with normal parents [8, 19, 39]. The disease has been seen in a wide ethnic and geographic distribution, but more commonly in Italians. A registry has been developed in Italy [42]. Heterozygote detection and prenatal diagnosis have not been reported, but this should be possible in those families in which the mutation is known.

A fluorometric procedure developed permitted the initiation of programs of neonatal screening for elevated concentrations of tyrosine [44]. These have been supplanted by the tandem mass spectrometry (MS/MS) programs of expanded neonatal screening.

An animal model for oculocutaneous tyrosinemia is available in mink, where it produces a disorder known as 'pseudodistemper' in which there are exudative lesions of the eyes and volar skin [45]. The activity of tyrosine aminotransferase is reduced, and there is a reduced amount of hepatic cytosolic immunoreactive protein in these animals. There is also a canine model in German Shepherds [46].

Tyrosine aminotransferase normally converts tyrosine to p-hydroxyphenylpyruvic acid (Figure 20.1). It is the rate-limiting step in the metabolism of tyrosine. The enzyme is highly regulated. Transcription is induced by glucocorticoids and cyclic AMP [47]. It is also developmentally regulated and human neonatal levels of activity are low [48]. The enzyme is a dimer, that is phosphorylated and acetylated at its N-terminus. Pyridoxal phosphate is bound to lysine [49]. The activity of the enzyme has been measured in liver of patients [1, 15, 29, 50, 51] and found to be low.

The gene for TAT has been sequenced in human [18], mouse [52], and rat [53]. It contains 12 exons spanning 10.9 kb. The mRNA is 2.75 kb. The 50.4 kDa protein has 454 amino acids. A rearrangement in the structural gene of one patient was demonstrated by Southern blot analysis [17]. Among a number of point mutations reported [54, 55], an R57X is frequent in Italian patients. Two missense mutations, G362V and R4331Y, converted a glycine to a valine and an arginine to an asparagine. There were two splice-site mutations and three conversions to stop codons. To date, it has not been possible to correlate phenotype with genotype.

When the activity of the enzyme is deficient, tyrosine accumulates, and tyrosine is the only amino acid that accumulates. Reported levels in the blood have generally ranged between 1100 and 3300 $\mu\text{mol/L}$ (20 and 50 mg/dL) (Table 20.1) [1, 12, 21, 24, 42, 56]. A level of 1000 $\mu\text{mol/L}$ appears to be a threshold level below which symptoms do not occur [2, 34]. Younger patients may have higher levels and this is consistent with higher intakes of protein. We have observed higher levels during winter than in summer and could correlate this with a decrease in protein consumption in summer, even in San Diego, and despite no change in the diet prescribed, or in symptoms [2]. Prior to diagnosis, the mother had noted that symptoms regularly improved during the summer. Some patients have avoided protein-containing foods [19].

The tyrosinemia in this disorder is generally considerably greater than in other forms of tyrosinemia. Furthermore, analysis of the amino acids of the blood permits its distinction from transient tyrosinemia of the newborn because concentrations of other amino acids, particularly phenylalanine and methionine, are not elevated. It may be distinguished from hepatorenal tyrosinemia by the absence of a generalized aminoaciduria.

In oculocutaneous tyrosinemia, the excretion of tyrosine in the urine is increased to 180 and 2000 mmol/mol creatinine [4, 13, 26]. Acetyltyrosine is also found in the urine [13] when serum concentrations of tyrosine exceed 2500 $\mu\text{mol/L}$. Tyrosine may be converted to tyramine, and this compound may be found in the urine

Table 20.1 Oculocutaneous tyrosinemia

	Plasma tyrosine concentrations ($\mu\text{mol/L}$)
Untreated patients on diagnosis	1100–2800
KP: ad lib diet – no sx	1215
KP: max during 11 months with no symptoms	1099
JF: asymptomatic	1073
Normal newborn	25–103
Child/adult	30–90

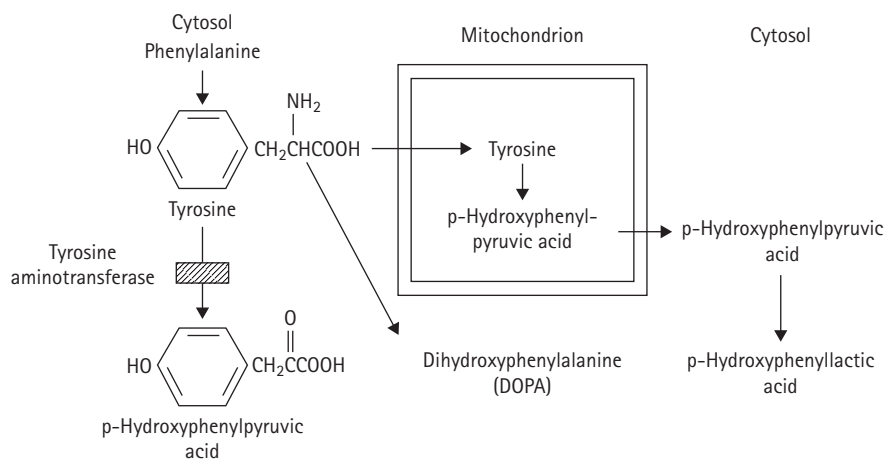


Figure 20.8 Metabolic interrelations in deficiency of hepatic cytosolic tyrosine aminotransferase. The site of the defect is on the left. The mechanism for the excretion of p-hydroxyphenylpyruvic acid is illustrated. Accumulated tyrosine becomes a substrate for the mitochondrial tyrosine aminotransferase which leads to the formation of p-hydroxyphenylpyruvic acid. In liver, this compound is readily converted to homogentisic acid and further oxidized, but this enzyme is widely distributed among other tissues of the body, and therefore p-hydroxyphenylpyruvic acid accumulates and is excreted in the urine. (Reprinted with permission from Nyhan WL. *Abnormalities in Amino Acid Metabolism in Clinical Medicine*. Norwalk, CT: Appleton-Century-Crofts, 1984.)

[13, 14]. Elevated concentrations of tyrosine are also found in the cerebrospinal fluid. Levels of 190–450 $\mu\text{mol/L}$ have been reported [27, 33, 36].

Analysis of the organic acids of the urine reveals large amounts of p-hydroxyphenylpyruvic acid, p-hydroxyphenyllactic acid, and p-hydroxyphenylacetic acid. The excretion of large amounts of p-hydroxyphenylpyruvic acid and p-hydroxyphenyllactic acid in the urine seems at first to be inconsistent with the site of the metabolic block. It is explained (Figure 20.8) by the widespread distribution of the other transaminase, mitochondrial tyrosine aminotransferase (aspartate aminotransferase, EC 2.6.1.1), in tissues other than liver, which lack the hydroxylase that catalyzes the conversion of p-hydroxyphenylpyruvic acid to homogentisic acid [57]. Accumulated tyrosine found in the blood is converted to p-hydroxyphenylpyruvic acid in tissues such as muscle. This compound is readily reduced to p-hydroxyphenyllactic acid [58]. Both p-hydroxyl compounds are then transported in the blood to the kidney, where they are effectively cleared and excreted in the urine [59].

TREATMENT

The treatment of oculocutaneous tyrosinemia consists of the institution of a diet low in tyrosine and phenylalanine. This effectively lowers concentrations of tyrosine in body fluids. Clinical symptomatology promptly resolves. Preparations (Tyrex (Ross) and Xphe XtyrAnalog, Maxamaid (SHS)) are available which are low in tyrosine and phenylalanine and simplify the preparation of formulas for the feeding of infants with tyrosinemia. Attention to

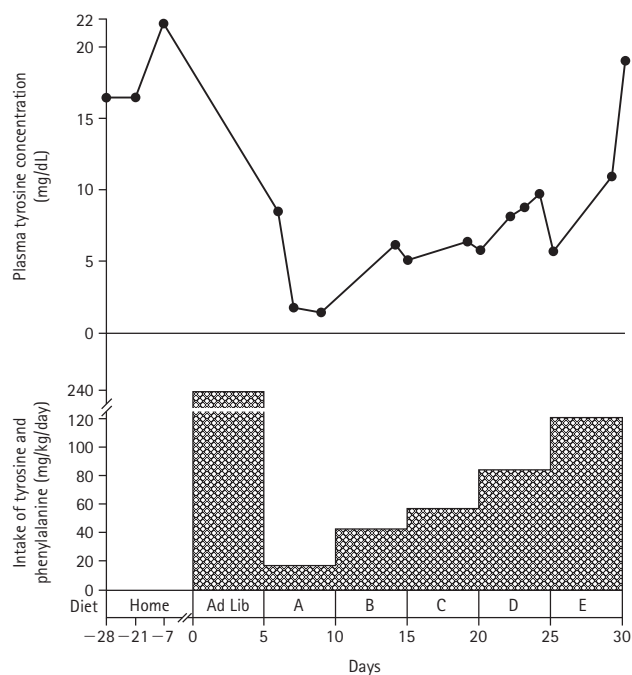


Figure 20.9 Relation of the plasma concentration of tyrosine to the intake of tyrosine and phenylalanine. (Reprinted with permission from Nyhan WL. *Abnormalities in Amino Acid Metabolism in Clinical Medicine*. Norwalk, CT: Appleton-Century-Crofts, 1984.)

compliance is important because treatment can prevent permanent ocular damage. The fact that symptoms of the disorder may be quite uncomfortable assists in the compliance of older patients. Whether early therapy

prevents impaired mental development is not clear, but early dietary management is prudent. There is excellent correlation between the concentration of tyrosine in the plasma and the intake of the amino acid and its precursor (Figure 20.9). Reasonable levels of control and an absence of symptoms are readily achieved using acceptable diets in childhood, as well as in infancy.

Optimal blood levels have not been defined, but most patients are free of symptoms as long as the plasma concentration of tyrosine is below 550–700 $\mu\text{mol/L}$ [2, 60–62]. Treatment with oral etretinate has been reported to improve skin lesions without changing levels of tyrosine [35], but this seems less than desirable because the skin is usually easier to control than the eye. Occasional noncompliance is not a problem as long as control is maintained most of the time.

Experience with maternal tyrosinemia indicates that dietary control during pregnancy would be prudent [43]. On the other hand, a number of normal offspring of mothers with this disease have been recorded [21, 42], so control of tyrosine levels can be rewarding.

REFERENCES

- Campbell RA, Buist NRM, Jacinto EY *et al.* Supertyrosinemia (tyrosine transaminase deficiency) congenital anomalies and mental retardation. *Proc Soc Pediatr Res* 1967; **37**: 80.
- Ney D, Bay C, Schneider JA *et al.* Dietary management of oculocutaneous tyrosinemia in an eleven-year-old. *Am J Dis Child* 1983; **137**: 995.
- Bardelli AM, Borgogni P, Farnetani MA *et al.* Familial tyrosinaemia with eye and skin lesions. *Ophthalmology* 1977; **175**: 5.
- Goldsmith LA, Reed J. Tyrosine-induced eye and skin lesions. *J Am Med Assoc* 1976; **236**: 382.
- Sandberg HD. Bilateral keratopathy and tyrosinosis. *Acta Ophthalmol* 1975; **53**: 760.
- Garibaldi LR, Siliato F, De Martini I *et al.* Oculocutaneous tyrosinosis. Report of two cases in the same family. *Helv Paediatr Acta* 1977; **32**: 173.
- Charlton KH, Binder PS, Wozniak L, Digby DJ. Pseudodendritic keratitis and systemic tyrosinemia. *Ophthalmology* 1981; **88**: 355.
- Hunziker N. Richner-Hanhart syndrome and tyrosinemia type II. *Dermatologica* 1980; **160**: 180.
- Richner H. Hornautaffektion bei Keratoma palmare et plantare hereditarium. *Klin Monatsbl Augenheilkd* 1938; **100**: 580.
- Hanhart E. Neue Sonderformen von Keratosis palmoplantaris ua eine regelmaessigdominante mit systematisieren Lipomen ferner 2 einfach-rezessive mit Schwachsinn zt mit Hornhautveraenderungen des Auges. *Dermatologica* 1947; **94**: 286.
- Kennaway NG, Buist NMR. Metabolic studies in a patient with hepatic cytosol tyrosine aminotransferase deficiency. *Pediatr Res* 1971; **5**: 287.
- Fellman JH, Buist NR, Kennaway NG, Swanson RE. The source of aromatic ketoacids in tyrosinemia and phenylketonuria. *Clin Chim Acta* 1972; **39**: 243.
- Kennaway NG, Buist NR. Metabolic studies in a patient with hepatic cytosol tyrosine aminotransferase deficiency. *Pediatr Res* 1971; **5**: 287.
- Hill A, Zaleski WA. Tyrosinosis: biochemical studies of an unusual case. *Clin Biochem* 1971; **4**: 263.
- Fellman JH, Vanbellinghen PJ, Jones RT, Koler IRD. Soluble and mitochondrial forms of tyrosine aminotransferase. Relationship to human tyrosinemia. *Biochemistry* 1969; **8**: 615.
- Barton DE, Yang-Feng TL, Francke U. The human tyrosine aminotransferase gene mapped to the long arm of chromosome 16 (region 16q22–q24) by somatic cell hybrid analysis and *in situ* hybridization. *Hum Genet* 1986; **72**: 221.
- Natt E, Westphal EM, Toth-Fejel SE *et al.* Inherited and *de novo* deletion of the tyrosine aminotransferase gene locus at 16q221q223 in a patient with tyrosinemia type II. *Hum Genet* 1987; **77**: 352.
- Rettenmeier R, Natt E, Hanswalter Z, Scherer G. Isolation and characterization of the human tyrosine aminotransferase gene. *Nucleic Acids Res* 1990; **18**: 3853.
- Rabinowitz LG, Williams LR, Anderson CE *et al.* Painful keratoderma and photophobia: hallmarks of tyrosinemia type II. *J Pediatr* 1995; **126**: 266.
- Gounod N, Ogier H, Dufier J-L *et al.* Tyrosinose oculo-cutanée de type II. *Ann Dermatol Venerol* 1984; **111**: 697.
- Chitayat D, Balbul A, Hani V *et al.* Hereditary tyrosinemia type II in a consanguineous Ashkenazi Jewish family: intrafamilial variation in phenotype; absence of parental phenotype effects on the fetus. *J Inherit Metab Dis* 1992; **15**: 198.
- Zammarchi E, La Cauza C, Calzolari C. Un caso di ipertyrosinemia con tirosiluria. *Minerva Pediatr* 1974; **26**: 203.
- Heidemann DG, Dunn SP, Bawle EV *et al.* Early diagnosis of tyrosinemia type II. *Am J Ophthalmol* 1989; **107**: 559.
- Colditz PB, Yu JS, Billson FA *et al.* Tyrosinemia II. *Med J Aust* 1984; **141**: 244.
- Bienfang DC, Kuwabara T, Pueschel SM. The Richner-Hanhart syndrome. Report of a case with associated tyrosinemia. *Arch Ophthalmol* 1976; **94**: 1133.
- Patton TH, Hosty TS. Tyrosinosis: a patient without liver or renal disease. *Pediatrics* 1971; **48**: 393.
- Pelet B, Antener I, Faggioni R *et al.* Tyrosinemia without liver or renal damage with plantar and palmar keratosis and keratitis (hypertyrosinemia type II). *Helv Paediatr Acta* 1979; **34**: 177.
- Faull KF, Gan I, Halpern B *et al.* Metabolic studies on two patients with nonhepatic tyrosinemia using deuterated tyrosine loads. *Pediatr Res* 1977; **11**: 631.
- Billson FA, Danks DM. Corneal and skin changes in tyrosinaemia. *Aust J Ophthalmol* 1975; **3**: 112.
- Boctor AM, Harper AE. Tyrosine toxicity in the rat: effect of high intake of p-hydroxyphenylpyruvic acid and of force-feeding high tyrosine diet. *J Nutr* 1968; **95**: 535.
- Goldsmith LA. Tyrosinemia II: lessons in molecular pathophysiology. *Pediatr Dermatol* 1983; **1**: 25.
- Driscoll DJ, Jabs EW, Alcorn D *et al.* Corneal tyrosine crystals in transient neonatal tyrosinemia. *J Pediatr* 1988; **113**: 91.

33. Goldsmith LA, Kng E, Bienfang DC *et al.* Tyrosinemia with plantar and palmar keratosis and keratitis. *J Pediatr* 1973; **83**: 798.
34. Al-Essa M, Rashed M, Ozand PT. Tyrosinemia type II: report of the first four cases in Saudi Arabia. *Ann Saudi Med* 1998; **18**: 466.
35. Fraser NG, MacDonald J, Griffiths WA *et al.* Tyrosinemia type II (Richner-Hanhart syndrome): report of two cases treated with etritinate. *Clin Exp Dermatol* 1987; **12**: 440.
36. Larreque M, Giacomoni DE, Bressieux J-M, Grilevre M. Syndrome de Richner-Hanhart ou tyrosinose oculo-cutanée. *Ann Dermatol Veneréol (Paris)* 1979; **106**: 53.
37. Bohnert A, Anton-Lamprecht I. Richner-Hanhart's syndrome: ultrastructural abnormalities of epidermal keratinization indicating a causal relationship to high intracellular tyrosine levels. *J Invest Derm* 1982; **79**: 68.
38. Zaleski WA, Hills A, Kushnikuk W. Skin lesions in tyrosinosis: response to dietary treatment. *Br J Dermatol* 1973; **88**: 335.
39. Goldsmith LA, Thorpe JM, Roe CR. Hepatic enzymes of tyrosine metabolism in tyrosinemia II. *J Invest Dermatol* 1979; **73**: 530.
40. Callan NJ. Circumscribed palmoplantar keratoderma. *Aust J Dermatol* 1970; **11**: 76.
41. Westmore R, Billson FA. Pseudoherpetic keratitis. *Br J Ophthalmol* 1973; **57**: 654.
42. Fois A, Borgogni P, Cioni M *et al.* Presentation of the data of the Italian registry for oculocutaneous tyrosinemia. *J Inherit Metab Dis* 1986; **9**: 262.
43. Cerone R, Fantasia AR, Castellano E *et al.* Pregnancy and tyrosinemia type II. *J Inherit Metab Dis* 2002; **25**: 317.
44. Grenier A, Laberge C. A modified automated fluorometric method for tyrosine determination in blood spotted in paper: a mass screening procedure for tyrosinemia. *Clin Chem Acta* 1974; **55**: 41.
45. Goldsmith LA, Thorpe JM, Marsh RF. Tyrosine aminotransferase deficiency in mink (*Mustela vison*): a model for human tyrosinemia II. *Biochem Genet* 1981; **19**: 687.
46. Kunkle GA, Jczyk PF, West CS *et al.* Tyrosinemia in a dog. *J Am Anim Hosp Assoc* 1984; **20**: 615.
47. Granner DK, Hargrove JL. Regulation of the synthesis of tyrosine aminotransferase: the relationship to mRNATAT. *Mol Cell Biochem* 1983; **53**: 113.
48. Hargrove JL, Mackin RB. Organ specificity of glucocorticoid-sensitive tyrosine aminotransferase isoenzymes. *J Biol Chem* 1984; **259**: 386.
49. Hargrove JL, Scoble HA, Matthews WR *et al.* The structure of tyrosine aminotransferase: evidence for domains involved in catalysis and enzyme turnover. *J Biol Chem* 1989; **264**: 45.
50. Lemmonier F, Charpentier C, Odievre M *et al.* Tyrosine aminotransferase isoenzyme deficiency. *J Pediatr* 1979; **94**: 931.
51. Kida A, Takahashi M, Fujisawa Y, Matsuda H. Hepatic tyrosine aminotransferase in tyrosinemia type II. *J Inherit Metab Dis* 1982; **5**: 229.
52. Muller G, Scherer G, Zentgraf H *et al.* Isolation characterization and chromosomal mapping of the mouse tyrosine aminotransferase gene. *J Mol Biol* 1985; **184**: 367.
53. Shinomoya T, Scherer G, Schmid W *et al.* Isolation and characterization of the rat tyrosine aminotransferase gene. *Proc Natl Acad Sci USA* 1984; **81**: 1346.
54. Natt E, Kida K, Odievre M *et al.* Point mutations in the tyrosine aminotransferase gene in tyrosinemia type II. *Proc Natl Acad Sci USA* 1992; **89**: 9297.
55. Huhn R, Stoermer H, Klingele B *et al.* Novel and recurrent tyrosine aminotransferase gene mutations in tyrosinemia type II. *Hum Genet* 1998; **102**: 305.
56. Armstrong MD, Stave U. A study of plasma free amino acid levels: II. Normal values for children and adults. *Metab Clin Exp* 1973; **22**: 561.
57. Kennaway NG, Buist NRM, Fellman JH. The origin of urinary p-hydroxy-phenylpyruvate in a patient with hepatic cytosol tyrosine aminotransferase deficiency. *Clin Chim Acta* 1972; **41**: 157.
58. Weber WW, Zannoni VG. Reduction of phenylpyruvic acids to phenyllactic acids in mammalian tissues. *J Biol Chem* 1969; **241**: 615.
59. Buist NRM, Kennaway NG, Fellman JH. Disorders of tyrosine metabolism. In: Nyhan WL (ed.). *Heritable Disorders of Amino Acid Metabolism*. New York: Wiley & Sons, 1974: 160.
60. Herve F, Moreno JL, Ogier H *et al.* Keratite inguerissable et hyperkeratose palmo-plantaire chronique avec hypertyrosinemia. *Arch Fr Pediatr* 1986; **43**: 19.
61. Machino H, Miki Y, Kawatsu T *et al.* Successful dietary control of tyrosinemia II. *J Am Acad Dermatol* 1983; **9**: 533.
62. Saijo S, Kudoh K, Kuramoto Y *et al.* Tyrosinemia II: report of an incomplete case and studies on the hyperkeratotic stratum corneum. *Dermatologica* 1991; **182**: 168.

Hepatorenal tyrosinemia/fumarylacetoacetate hydrolase deficiency

Introduction	171	Treatment	176
Clinical abnormalities	171	References	176
Genetics and pathogenesis	174		

MAJOR PHENOTYPIC EXPRESSION

Hepatocellular degeneration leading to acute hepatic failure, or chronic cirrhosis and hepatocellular carcinoma; renal Fanconi syndrome; peripheral neuropathy; hypertyrosinemia; succinylacetonuria; and deficiency of fumarylacetoacetate hydrolase.

INTRODUCTION

Hepatorenal tyrosinemia, which has been referred to as tyrosinemia type 1, tyrosinosis, or hereditary tyrosinemia, was first reported by Sakai and Kitagawa in 1957 [1–3]. The patient reported was the product of a consanguineous mating, who developed progressive liver disease which led to death with hematemesis and hepatic coma at three years of age. In addition, the patient had rickets, which was resistant to vitamin D. The major metabolic products in the urine were p-hydroxyphenyllactic acid, p-hydroxyphenylpyruvic acid, and p-hydroxyphenylacetic acid, as well as tyrosine. Gentz and colleagues [4], in a report of seven patients with the disease, first characterized the renal component as a Fanconi syndrome. It was noted that patients had neurologic crises reminiscent of porphyria [5, 6], and this led to the recognition that δ -aminolevulinic acid was excreted in large amounts [6–9]. Lindblad and colleagues [10] reported that succinylacetone, which they found in the urine of these patients, is an inhibitor of the synthesis of porphobilinogen from δ -aminolevulinic acid. They reasoned that the fundamental defect was in the activity of fumarylacetoacetate hydrolase (Figure 21.1). This was confirmed enzymatically by these investigators [11] and others [12–14].

The gene has been cloned [15, 16] and mapped to chromosome 15q23–25. Mutations have been identified

[17], including founder mutations in French-Canadian Quebec and in Finland, where the disease is prevalent. The Quebec mutation is a splice mutation IVS12+5G-A [18], and that in Finland is W262X [19]. The discovery of a therapeutic agent 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) (nitisinone) represents a major advance in the management of this disease [20, 21].

CLINICAL ABNORMALITIES

The clinical course of hepatorenal tyrosinemia has generally followed one of two patterns: an acute or a chronic form. The former has sometimes been referred to as the French-Canadian type and the latter the Scandinavian type, but of course there is considerable overlap [4, 5, 14, 22]. Most patients have had acute presentations. Symptoms develop in early infancy and they are those of acute hepatic decompensation. Hepatic failure and death occurs usually under one year of age. However, some infants with an acute onset of hepatic disease survive to go on to display a chronic disease just like those patients with the chronic form. The differential diagnosis of hepatic failure is given in the [Appendix](#). Until recently, most of these children died at younger than ten years of age. Only one patient of those described early survived to the age of 20 years [22]. The year 1 mortality for those presenting with symptoms by

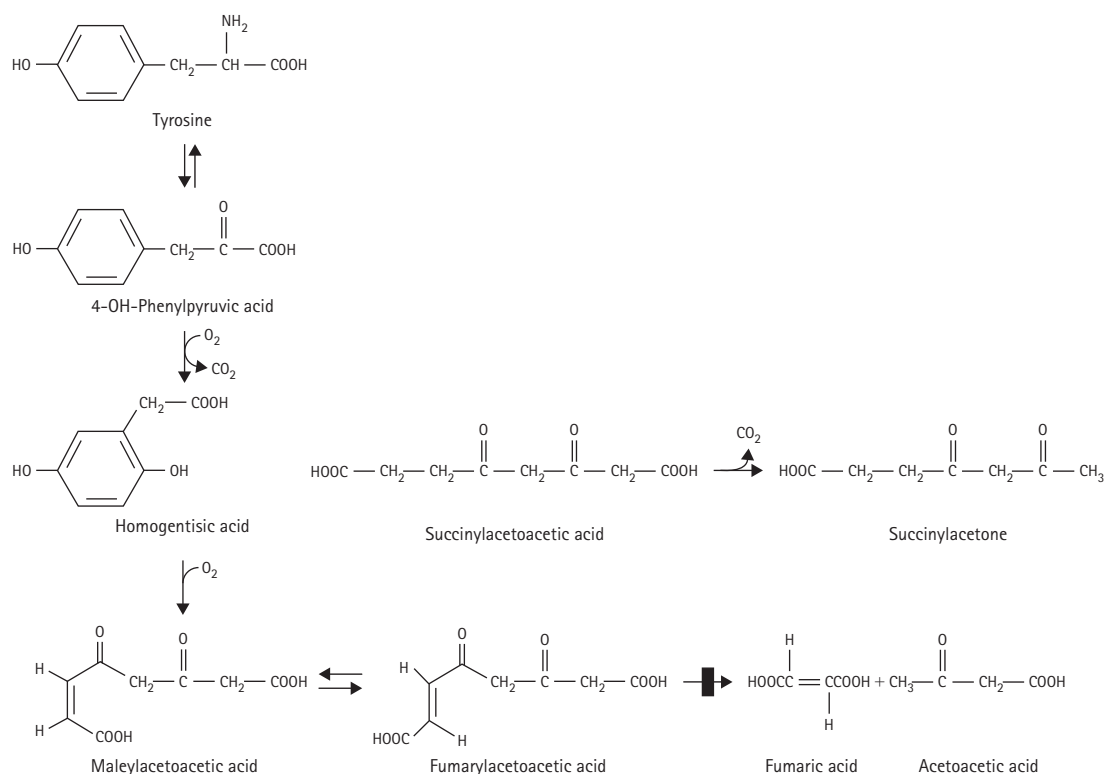


Figure 21.1 Metabolism of tyrosine and phenylalanine. The site of defect hepatorenal tyrosinemia is in fumarylacetoacetate hydrolase.



Figure 21.2 AY: A seven-year-old girl with hepatorenal tyrosinemia.

two months was 60 percent; of those presenting between two and six months, it was 20 percent; and of those presenting after six months it was 4 percent [23]. Prognosis has changed dramatically with currently available therapy.

The earliest and the major effect of the disease is on



Figure 21.3 AMQ: A boy with hepatorenal tyrosinemia. The abdominal enlargement resulted from the liver which was palpable 4 cm below the costal margin.

the liver. Abdominal distension and failure to thrive are prominent, and may be associated with vomiting and/or diarrhea (Figures 21.2, 21.3, and 21.4). The acute hepatic crisis is the most common early presentation. The infant may appear acutely or desperately ill and have jaundice and ascites along with hepatomegaly [24–26]. There may be gastrointestinal bleeding. Hypoglycemia may be a presenting symptom. Hepatic crises may be triggered by infection.

Several infants have been noted by the mothers to have a peculiar sweet odor. A boiled cabbage-like odor in some



Figure 21.4 JQ: A girl with hepatorenal tyrosinemia. She had cirrhosis with abdominal enlargement and ascites.

patients has been related to a metabolite of methionine 2-oxo-4-methiolbutyric acid [27–30].

Transaminase levels in the blood may be normal or slightly elevated. The rare elevation over 1000 IU/L indicates substantial damage to hepatic cells. α -Fetoprotein may be markedly elevated, ranging from 100,000 to 400,000 ng/mL. Coagulation factors may be abnormal and there may be bleeding. Prothrombin times and partial prothrombin times may be markedly elevated. Coagulopathy is characteristically unresponsive to vitamin K. Jaundice is uncommon early in this disease.

One of our patients presented with bleeding and was investigated as a problem in coagulation before chemical evidence of hepatic disease was identified. Patients may present with epistaxis or intestinal bleeding [31]. Elevated levels of prothrombin time (PT) and partial thromboplastin time (PTT) may be found even in asymptomatic infants discovered by newborn screening. An infant presenting with liver disease and hypoglycemia may be thought to have Reye syndrome. Between acute crises the liver is enlarged. α -Fetoprotein may be slightly or greatly elevated.

The chronic liver disease picture is that of hepatic cirrhosis. The differential diagnosis of hepatic cirrhosis in infancy is given in the [Appendix](#). The pathologic picture is that of macronodular cirrhosis [32]. Splenomegaly develops. There may be acute crises of increased hepatocellular damage, often precipitated by infection, and these may lead to hepatic failure. Esophageal varices may develop and they may be complicated by bleeding.

A more common complication is the development of hepatocellular carcinoma [33, 34]. The risk of this complication has been variously reported. In a series of 42 patients reported in 1976 [35] from the United States, 37 percent of those over two years of age developed carcinoma, while information from an international series yielded an incidence of 18 percent of those over two years [36]. Detection of nodules by computed tomography (CT) scan or ultrasound appears to be quite reliable, because histologic examination of 18 livers from patients subjected

to liver transplantation failed to reveal focal carcinomas in patients not found to have nodules by those modalities [37]. CT should be performed with and without contrast. Liver cancer has been documented as early as 33 months of age [38]. Even younger, a 15-month-old patient was found to have a carcinoma following a presentation at five months with acute hepatic failure and a good response to NTBC with a ten-fold drop in α -fetoprotein, which then rose to 100,000 ng/mL [39]. A significant rise in the level of α -fetoprotein may herald the onset of carcinoma, but carcinoma was found in a patient whose level was only 87 ng/mL [3]. Patients should be monitored regularly by CT, magnetic resonance imaging (MRI), or ultrasound, and nodules should be biopsied.

Renal disease is another characteristic feature of this disease. Among 32 patients [40], 47 percent had enlargement of the kidneys, often palpable [31]; 47 percent had increased echogenicity of the kidneys and 16 percent had nephrocalcinosis. In another eight patients [41], 50 percent had nephromegaly. The renal tubular disease is that of a typical renal Fanconi syndrome in which there is phosphaturia, aminoaciduria, and often glycosuria. There

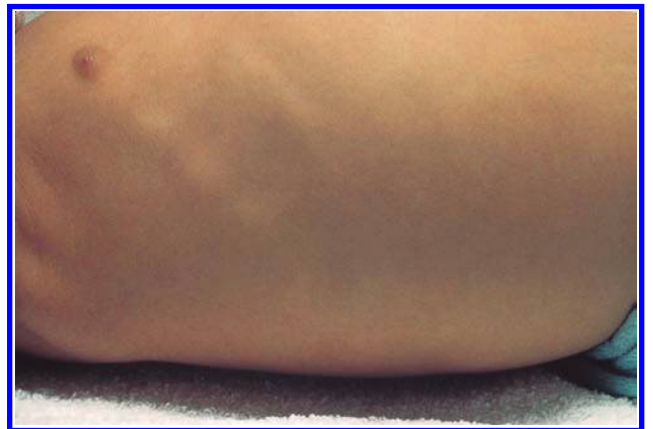


Figure 21.5 AY: Illustrating the rachitic rosary.



Figure 21.6 AY: The wrist was enlarged because of rachitic changes at the ends of bones.



Figure 21.7 AMQ: The wrist was also enlarged.

may be proteinuria. Systemic metabolic acidosis may result from renal tubular dysfunction. The phosphate losses lead to hypophosphatemia and clinical rickets (Figures 21.5, 21.6, and 21.7). There may also be a variable reduction in glomerular function. In the series of 32 patients [40], 48 percent had decreased glomerular filtration; 82 percent had aminoaciduria, 67 percent hypercalcinuria, and 59 percent renal tubular acidosis. Affected infants have been observed to have vitamin D-resistant rickets at less than four months [25], which is unusual.

Neurologic crises of pain and paresthesia are a result of peripheral neuropathy [42–45]. These may occur in as many as 42 percent of patients. Crises may be mistaken for porphyria [43]. There may be extensor hypotonus or the patient may have hypertonia. Systemic, autonomic signs include hypertension, tachycardia, and ileus. Pain usually begins in the legs. The patient may position the head and trunk in extreme hyperextension and may be thought to have opisthotonus or meningismus [29]. Muscular weakness may progress to paralysis requiring artificial ventilation [42]. Self-injurious behavior has been observed. Some patients have had seizures [43], some of them associated with hyponatremia [8]. Death may occur during a neurologic crisis [44, 45]. During most crises, consciousness is normal. These crises are not associated with hepatic relapse. Most crises subside in 1–7 days and resolve slowly, but there may be residual weakness. Intelligence is usually normal.

Three infants have had obstructive hypertrophic cardiomyopathy [46, 47] and this may be fatal [46]. Two patients have had macroglossia [7, 36] and there may be macrosomia. Pancreatic islet hypertrophy is common, but usually asymptomatic. Hypoglycemia can usually be attributed to hepatic disease.

GENETICS AND PATHOGENESIS

Hepatorenal tyrosinemia is transmitted in an autosomal recessive fashion [26, 48]. Consanguinity has been

documented in a number of families [49]. A particularly high frequency of 1.46 per 1000 births has been recorded in a French-Canadian isolate in the Chicoutimi–Lac St Jean region of northeastern Quebec [49, 50], where the carrier rate is one in 20 [47]. An overall incidence of 0.8 per 10,000 births was observed in the French-Canadian population of Quebec. The prevalence has approximated at 1 in 100,000 [51] from newborn screening programs in Scandinavia. Founder effects have been elucidated in the French-Canadian population [52, 53]. The disease is frequent in French-Canada and relatively so in Scandinavia, but it may be found in any geographic or ethnic background.

The molecular defect in hepatorenal tyrosinemia is in the hepatic fumarylacetoacetic acid hydrolase (fumarylacetoacetase, EC 3.7.1.2) (Figure 21.1). This was originally proposed on the basis of the accumulation of succinylacetone [10]. Deficiency of this enzyme was then documented by assay of activity in liver [12]. The level was 6 percent of normal in six patients with the acute disease and 20 percent in two patients with the chronic form. The activity of maleylacetoacetic acid hydrolase was also deficient in some samples of liver. A problem with enzyme assay is that in the presence of liver disease, the activity of many enzymes is reduced, but the enzyme deficiency may also be demonstrated in lymphocytes and fibroblasts [54]. The gold standard in the diagnosis of this disease is the demonstration of succinylacetone in the urine.

Heterozygote detection has been carried out by the assay of fumarylacetoacetate hydrolase activity in fibroblasts and lymphocytes [55]. Obligatory heterozygotes have had a mean level that is 50 percent of normal, but considerable variation and the possibility of pseudoalleles make this unreliable. Where the mutation is known or in populations like that of Quebec where a small number of mutations is responsible, molecular testing is the preferred method. Prenatal diagnosis has been accomplished by assay of the enzyme in cultured amniocytes or chorionic villus material [55–57]. It has also been accomplished by the direct assay of concentrations of succinylacetone in amniotic fluid [58, 59], and this is thought to be the method of choice. However, at least one affected infant has been missed in this assay [60]. In families in which the mutation is known, molecular methods are ideal.

The gene has been localized to chromosome 15q23-25 [15]. The gene contains 14 exons over a span of 30–34 kb [61]. A number of restriction fragment length polymorphisms

Table 21.1 Variants of fumarylacetoacetate hydrolase in hepatorenal tyrosinemia

Type	mRNA	CRM	Enzyme activity
A	11	0	0
B	1	1	1
C	11	11	0
D	0	0	0

Fumarylacetoacetate hydrolase		
	Control	Patient
DNA (47)	CAA	CTA
Enzyme (16)	Asparagine	Isoleucine

Figure 21.8 Variant fumarylacetoacetate hydrolase gene and enzyme in a French-Canadian patient with hepatorenal tyrosinemia. The numbers in parentheses indicate nucleotide 47 in the gene and amino acid 16 in the protein.

(RFLP) have been identified, and these RFLPs may be used for carrier detection and prenatal diagnosis [62]. Ten haplotypes were found with five RFLPs in the French-Canadian population. Haplotype 6 was strongly associated with the disease; its frequency was 90 percent in French-Canadian and 96 percent in Saguenay–Lac-Saint-Jean. A considerable number and variety of mutations have been identified (Table 21.1), and heterogeneity has been identified, even in the French-Canadian population [61, 63]. In a French-Canadian patient, an A-to-T transversion changed an asparagine to isoleucine at position 16 (Figure 21.8) [17]. The IVS12+5G-A mutation is more common [18]. In a Norwegian patient, a missense mutation changed alanine at 134 to aspartic acid [61]. A splice-site mutation resulting from a G-to-A transition was found to lead to deletion of exon 12 in a French-Canadian patient [64]. Among 62 patients of varied ethnicity the IVS12+5G-A+5, the common French-Canadian mutation, was the most common found in 32 alleles from the United States, Europe, Pakistan, and Turkey [19]. A Scandinavian mutation c.1009G>A was a splice mutation, as was c.192G>T found in Pakistanis. Splice-site mutations were also common in 92.8 percent of alleles among 29 patients from the Mediterranean area [65]; IVS6-1G-T was the most common. A pseudodeficiency allele p.R341W has been found in normal individuals with low activity of the hydrolase enzyme [66]. Mutations have been shown to produce mRNA without enzyme activity or cross-reacting material (CRM); mRNA and CRM without enzyme activity; mRNA, CRM, and some activity; as well as no mRNA. Patients with early onset hepatic failure tend to be CRM-negative.

The deficient enzyme is on the catabolic pathway for tyrosine, and this is the cause of the hypertyrosinemia (Figure 21.1). Fumarylacetoacetate accumulates and is converted to succinylacetoacetate and to succinylacetone. In hepatorenal tyrosinemia, concentrations of tyrosine usually range from 170 to 660 $\mu\text{mol/L}$ (3–12 mg/dL).

Increased quantities are also excreted in the urine. Of the tyrosyl compounds found in the urine, p-hydroxyphenyllactic acid is the most prominent; p-hydroxyphenylpyruvic acid and p-hydroxyphenylacetic acid are also present in appreciable quantities. Patients often have elevated concentrations of methionine in the

blood. Hypoglycemia is common, especially in the acute illness. In chronic cirrhosis or after treatment, tyrosine concentrations may be normal. On the other hand, during the acute stages of hepatocellular damage many other amino acids may be found in elevated amounts in the serum, including cystathionine, proline and hydroxyproline. These patterns, along with the tyrosine, are reflected in the urinary excretion of amino acids. They are superimposed on the generalized aminoaciduria that results from the renal tubular aspects of the disease. Patients also have phosphaturia and hypophosphatemia. The presence of reducing substance completes the picture of the renal Fanconi syndrome. The sugar is usually glucose, but other sugars have been reported [4, 67]. With progression, there is systemic acidosis, increased potassium loss, and hypokalemia.

The urinary excretion of δ -aminolevulinic acid is increased [8, 10, 68, 69]. Succinylacetoacetic acid and succinylacetone are found in the serum and the urine [10, 68], the direct consequence of the defective activity of fumarylacetoacetic acid hydrolase. Accumulated fumarylacetoacetic acid is reduced to succinylacetoacetic acid and decarboxylated to form succinylacetone. Succinylacetone has immunosuppressive activity [69]. It is also a powerful inhibitor of δ -aminolevulinic acid dehydratase [70], accounting for the increased excretion of δ -aminolevulinic acid and inhibition of the synthesis of porphobilinogen from δ -aminolevulinic acid. Succinylacetone can be found in spots of blood dried on filter paper. Screening for hepatorenal tyrosinemia has been undertaken in a number of states and countries. It has recently been incorporated into expanded tandem mass spectrometry (MS/MS) programs with tyrosine as the key analyte. This has the problem that transient tyrosinemia triggers a positive screen, and the numbers are such that many programs have set the screen level so high that most patients with hepatorenal tyrosinemia would be missed. In addition, some patients with this disease have normal levels of tyrosine. Quebec now screens for succinylacetone. A liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantification of succinylacetone in dried blood spots was developed for use in a two-tier screening method for this disease [71]. It could also be useful in monitoring treatment in patients. A combined assay for succinylacetone, amino acids, and acylcarnitine has since been developed [72] which required a separate extraction step not a single MS/MS.

The pattern of laboratory findings in this disease is virtually unique. A combination of hypoglycemia, coagulopathy, tyrosinemia, succinylacetone, and very high α -fetoprotein is diagnostic.

Fumarylacetoacetate is an inhibitor of methionine adenosyltransferase, and this would lead to hypermethioninemia [73], but methionine levels also increase nonspecifically in hepatocellular disease. Renal tubular dysfunction is thought to result from maleylacetoacetic acid by analogy with maleic acid, which can produce

an experimental Fanconi syndrome in animals [74]. Fumarylacetoacetate and maleylacetoacetate react with sulfhydryl compounds, and deficiency of glutathione has been documented in this disease [75]. Maleylacetone and succinylacetone can form glutathione adducts [76]. The accumulated products are highly reactive and can form stable adducts through the formation of Schiff bases with proteins and amino acids such as lysine for, and by the alkylation of thiols and amino groups, and this could be a mechanism for production of disease. The acute porphyria-like episodes of peripheral neuropathy in this disease are thought to result from the inhibition by succinylacetone of δ -aminolevulinic acid hydrolase and the formation of porphobilinogen.

An interesting phenomenon in this disease is the occurrence of revertant nodules in which hydrolase activity is normal [77, 78]. The enzyme protein is present in these nodules in which at least one allele has mutated to the normal sequence. This, of course, could lead to a finding of normal activity in biopsied liver. This reversal to the normal genotype involved the reversion of a mutant AT nucleotide pair to GC the normal and it was found in three different disease-producing mutations including the common splice-site mutation. In another example of mosaicism of normal and mutant phenotypes in patient liver, a new mutation upstream of the primary mutation suppressed the abnormal splicing [79].

TREATMENT

Treatment of this disease has been revolutionized by the discovery of NTBC (Figure 21.9) [20, 80]. Restriction of the dietary intake of phenylalanine and tyrosine will lower concentrations of tyrosine, and improvement in renal tubular function has been reported [35, 81–84]. Coagulation problems are also responsive. However, hepatic disease may progress despite dietary treatment.

Acute hepatic dysfunction must be treated aggressively. Energy and nutrition may be provided parenterally, as well as the management of fluids and electrolytes. Intake of phenylalanine and tyrosine is stopped temporarily. In liver failure, transplantation of a liver is the only answer. In a neurologic crisis, attention to respiration and assistance when necessary are mandatory.

Transplantation has also become the treatment

choice for hepatocellular carcinoma [85, 86]. In recent years, transplantation has been undertaken prior to the development of nodules in order to prevent carcinoma [87–89]. Survival rates at 36 months following transplantation of the liver for this disease have been as high as 87 percent [89]. Tyrosyl compounds in the urine decreased to normal, while succinylacetone decreased, but as far as normal in only one patient [90]. Presumably, this succinylacetone is made in the kidney. The management of a patient with multiple hypodense hepatic nodules has become complex because of a report that nodules might represent nodular cirrhosis and fatty change [91], and a report that with medical treatment such lesions disappeared [92].

Excretion of δ -aminolevulinic acid also decreased, but remained somewhat elevated. Renal tubular reabsorption of phosphate and bicarbonate may become normal within 5 days of transplantation; glycosuria and aminoaciduria correct within 2 weeks [93].

The advent of therapy with NTBC has changed the readiness with which hepatic transplantation is performed in this disorder. The indication now is hepatic cancer. NTBC is a potent inhibitor of p-hydroxyphenylpyruvate dioxygenase [94]. Treatment with 1 mg/kg of this compound has led regularly to improvement in hepatic and renal function, and no side effects have been observed. Concentrations of succinylacetone and α -fetoprotein have decreased, and hepatic morphology has improved. Excretion of δ -aminolevulinic acid has decreased to near normal, and erythrocyte porphobilinogen synthesis increased. This appears to eliminate the neurologic crises of the disease in those properly treated [95]. NTBC has been approved by the US Food and Drug Administration (FDA) for the treatment of hepatorenal tyrosinemia. As of 2003, 369 patients had been treated [96], and treatment was continuing on 293. Withdrawals were 76, of whom 26 had died; 21 had liver failure, of whom 12 died; 25 had developed hepatocellular carcinoma, of whom seven had died; and 54 had been transplanted, of whom eight died. Prior to NTBC, survival curves in this disease indicated few long-term survivors. Now, approximately 90 percent of those diagnosed before two years of age are alive, some as long as 12 years. The figure for those diagnosed late approximates 60 percent surviving 6–12 years. There have been only three hepatic cancers in those treated before two years of age, and one of these was present at diagnosis, before treatment. Improvement has been reported when NTBC was given during a neurologic crisis [96].

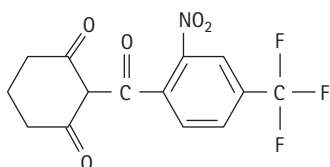


Figure 21.9 NTBC, 2(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione.

REFERENCES

1. Sakai K, Kitagawa T. An atypical case of tyrosinosis. Part 1. Clinical and laboratory findings. *Jikeikai Med J* 1957; 4: 1.
2. Sakai K, Kitagawa T. An atypical case of tyrosinosis Part 2. A research on the metabolic block. *Jikeikai Med J* 1957; 4: 11.
3. Sakai K, Kitagawa T, Yoshioka K. An atypical case of tyrosinosis Part 3. The outcome of the patient. *Jikeikai Med J* 1959; 6: 15.

4. Gentz J, Jagenburg R, Zetterstrom R. Tyrosinemia. *J Pediatr* 1965; **66**: 670.
5. Gentz J, Lindblad B, Lindstedt S *et al.* Dietary treatment in tyrosinemia (tyrosinosis). With a note on the possible recognition of the carrier state. *Am J Dis Child* 1967; **113**: 31.
6. Kang ES, Gerald PS. Hereditary tyrosinemia and abnormal pyrrole metabolism. *J Pediatr* 1970; **77**: 397.
7. Gaull GE, Rassin DK, Solomon GE *et al.* Biochemical observations on so-called hereditary tyrosinemia. *Pediatr Res* 1970; **4**: 337.
8. Strife CF, Zuroweste EL, Emmett EA *et al.* Tyrosinemia with acute intermittent porphyria: δ -aminolevulinic acid dehydratase deficiency related to elevated urinary aminolevulinic acid levels. *J Pediatr* 1977; **90**: 400.
9. Gentz J, Johansson S, Lindblad B *et al.* Excretion of delta-aminolevulinic acid in hereditary tyrosinemia. *Clin Chim Acta* 1969; **23**: 257.
10. Lindblad B, Lindstedt S, Steen G. On the enzymic defects in hereditary tyrosinemia. *Proc Natl Acad Sci USA* 1977; **74**: 4641.
11. Fällström S-P, Lindblad B, Lindstedt S, Steen G. Hereditary tyrosinemia-fumarylacetoacetase deficiency. *Pediatr Res* 1979; **13**: 78 (Abstr.).
12. Kvittingen EA, Jellum E, Stokke O. Assay of fumarylacetoacetate fumarylhydrolase in human liver-deficient activity in a case of hereditary tyrosinemia. *Clin Chim Acta* 1981; **115**: 311.
13. Berger R, Smit GP, Stoker-de Vries SA *et al.* Deficiency of fumarylacetoacetic in a patient with hereditary tyrosinemia. *Clin Chim Acta* 1981; **114**: 37.
14. Gray RG, Patrick AD, Preston FE, Whitfield MF. Acute hereditary tyrosinaemia type I: clinical biochemical and haematological studies in twins. *J Inherit Metab Dis* 1981; **4**: 37.
15. Phaneuf D, Labelle Y, Bérubé D *et al.* Cloning and expression of the cDNA encoding human fumarylacetoacetate hydrolase the enzyme deficient in hereditary tyrosinemia: assignment of the gene to chromosome. *Am J Hum Genet* 1991; **48**: 525.
16. Agsteribbe E, van Faassen H, Hartog MV *et al.* Nucleotide sequence of cDNA encoding human fumarylacetoacetase. *Nucleic Acids Res* 1990; **18**: 1887.
17. Phaneuf D, Lambert M, Laframboise R *et al.* Type I hereditary tyrosinemia. Evidence for molecular heterogeneity and identification of a causal mutation in a French Canadian patient. *J Clin Invest* 1992; **90**: 1185.
18. Grompe M, St-Louis M, Demers SI *et al.* A single mutation of the fumarylacetoacetate hydrolase gene in French Canadians with hereditary tyrosinemia type I. *N Engl J Med* 1994; **331**: 353.
19. Rootwelt H, Hoie K, Berger R, Kvittingen EA. Fumarylacetoacetate mutations in tyrosinaemia type I. *Hum Mutat* 1996; **7**: 239.
20. Lindstedt S, Holme E, Lock EA *et al.* Treatment of hereditary tyrosinaemia type I by inhibition of 4-hydroxyphenylpyruvate dioxygenase. *Lancet* 1992; **340**: 813.
21. Holme E, Lindstedt S. Diagnosis and management of tyrosinemia type I. *Curr Opin Pediatr* 1995; **7**: 726.
22. Halvorsen S, Pande H, Loken AC, Gjessing LR. Tyrosinosis. A study of 6 cases. *Arch Dis Child* 1966; **41**: 238.
23. van Spronsen FJ, Thomasse Y, Berger R *et al.* Lifetime expectancy with dietary treatment in tyrosinemia type I: consequences for timing of liver transplantation. 29th Symposium of Society for the Study of Inborn Errors of Metabolism, London, 1991: poster 21.
24. Scriver CR, Larochelle J, Silverberg M. Hereditary tyrosinemia and tyrosyluria in a French Canadian geographic isolate. *Am J Dis Child* 1967; **113**: 41.
25. Kogut MD, Shaw KN, Donnell GN. Tyrosinosis. *Am J Dis Child* 1967; **113**: 47.
26. Laberge C. Hereditary tyrosinemia in a French Canadian isolate. *Am J Hum Genet* 1969; **21**: 36.
27. Cone TE Jr. Diagnosis and treatment: some diseases, syndromes, and conditions associated with an unusual odor. *Pediatrics* 1968; **41**: 993.
28. Gahl WA, Finkelstein JD, Mullen KD *et al.* Hepatic methionine adenosyltransferase deficiency in a 31-year-old man. *Am J Hum Genet* 1987; **40**: 39.
29. Perry TL, Hardwick DF, Dixon GH *et al.* Hypermethioninemia: a metabolic disorder associated with cirrhosis islet cell hyperplasia and renal tubular degeneration. *Pediatrics* 1965; **36**: 236.
30. Perry TL. Tyrosinemia associated with hypermethioninemia and islet cell hyperplasia. *Can Med Assoc J* 1967; **97**: 1067.
31. Bas AY, Kunak B, Ertan U *et al.* Tyrosinemia type 1: a case report. *Int Pediatr* 2003; **18**: 45.
32. Fritzell S, Jagenburg OR, Schnürer L-B. Familial cirrhosis of the liver renal tubular defects with rickets and impaired tyrosine metabolism. *Acta Paediatr Scand* 1964; **53**: 18.
33. Gentz J, Heinrich J, Lindblad B *et al.* Enzymatic studies in a case of hereditary tyrosinemia with hepatoma. *Acta Paediatr Scand* 1969; **58**: 393.
34. Barness L, Gilbert-Barness E. Pathological case of the month. Special feature. *Am J Dis Child* 1992; **146**: 769.
35. Wehnberg AG, Mize CE, Worthen HG. The occurrence of hepatoma in the chronic form of hereditary tyrosinemia. *J Pediatr* 1976; **88**: 434.
36. Mitchell GA, Lambert M, Tanguay RM. Hypertyrosinemia. In: Scriver CR, Beaudet AL, Sly WS *et al.* (eds). *The Metabolic and Molecular Bases of Inherited Disease*, 7th edn. New York: McGraw Hill, 1995: 1077.
37. Paradis K, Weber A, Seidman EG *et al.* Liver transplantation for hereditary tyrosinemia: the Quebec experience. *Am J Hum Genet* 1990; **47**: 338.
38. Mieses LA, Esquivel CO, Van Thiel DH *et al.* Liver transplantation for tyrosinemia. A review of 10 cases from the University of Pittsburgh. *Dig Dis Sci* 1990; **35**: 153.
39. Dionisi-Vici C, Boglino C, Marcellini M *et al.* Tyrosinemia type I with early metastatic hepatocellular carcinoma: combined treatment with NTBC chemotherapy and surgical mass removal (abstract). *J Inherit Metab Dis* 1997; **20**(Suppl. 1): 3.
40. Forget S, Patriquin BH, Dubois J *et al.* The kidney in children with tyrosinemia: sonographic CT and biochemical findings. *Pediatr Radiol* 1999; **29**: 104.
41. Laine J, Salo MK, Krogerus L *et al.* Nephropathy of tyrosinemia and its long-term outlook. *J Pediatr Gastroenterol Nutr* 1997; **24**: 113.
42. Mitchell G, Larochelle J, Lambert M *et al.* Neurologic crises in hereditary tyrosinemia. *N Engl J Med* 1990; **322**: 432.

43. Goulden KJ, Moss MA, Cole DE *et al.* Pitfalls in the initial diagnosis of tyrosinemia: three case reports and a review of the literature. *Clin Biochem* 1987; **20**: 207.
44. van Spronsen FJ, Thomasse Y, Smit GPA *et al.* Hereditary tyrosinemia type I: a new clinical classification with difference in prognosis on dietary treatment. *Hepatology* 1994; **20**: 1187.
45. Strife CF, Zuroweste EL, Emmet EA *et al.* Tyrosinemia with acute intermittent porphyria: δ -aminolevulinic acid dehydratase deficiency related to elevated urinary aminolevulinic acid levels. *J Pediatr* 1977; **90**: 400.
46. Lindblad B, Fällström SP, Höyer S *et al.* Cardiomyopathy in fumarylacetoacetase deficiency (hereditary tyrosinaemia): a new feature of the disease. *J Inher Metab Dis* 1987; **10**: 319.
47. Edwards MA, Green A, Colli A, Rylance G. Tyrosinaemia type I and hypertrophic obstructive cardiomyopathy. *Lancet* 1987; **1**: 437 (letter).
48. De Braekeleer M, Larochelle J. Genetic epidemiology of hereditary tyrosinemia in Quebec and in Saguenay-Lac-St-Jean. *Am J Hum Genet* 1990; **47**: 302.
49. Bergeron P, Laberge C, Grenier A. Hereditary tyrosinemia in the province of Quebec. Prevalence at birth and geographic distribution. *Clin Genet* 1974; **5**: 157.
50. Laberge C, Dallaire L. Genetic aspects of tyrosinemia in the Chicoutimi region. *Can Med Assoc J* 1967; **97**: 1099.
51. Halvorsen S. Screening for disorders of tyrosine metabolism. In: Bickel H, Guthrie R, Hammersen G (eds). *Neonatal Screening for Inborn Errors of Metabolism*. New York: Springer-Verlag, 1980: 45.
52. Laberge C. Hereditary tyrosinemia in a French Canadian isolate. *Am J Hum Genet* 1969; **21**: 36.
53. Bouchard G, Laberge C, Scriver C-R. Comportements démographiques et effets fondateurs dans la population du Québec (XVIIe-Xxe siècles). In: *Anonymous Société Belge de Demographic Historiens et Populations: Liber Amicorum Etienne Hélin*. Louvain-la-Neuve: Academia, 1992: 319.
54. Kvittingen EA, Halvorsen S, Jellum E. Deficient fumarylacetoacetate fumarylhydrolase activity in lymphocytes and fibroblasts from patients with hereditary tyrosinemia. *Pediatr Res* 1983; **17**: 541.
55. Kvittingen EA, Brodtkorb E. The pre- and post-natal diagnosis of tyrosinemia type I and the detection of the carrier state by assay of fumarylacetoacetase. *Scand J Clin Lab Invest Suppl* 1986; **184**: 35.
56. Kvittingen EA, Guibaud PP, Divry P *et al.* Prenatal diagnosis of hereditary tyrosinaemia type 1 by determination of fumarylacetoacetase in chorionic villus material. *Eur J Pediatr* 1986; **144**: 597 (letter).
57. Kvittingen EA, Steinmann B, Gitzelmann R *et al.* Prenatal diagnosis of hereditary tyrosinemia by determination of fumarylacetoacetase in cultured amniotic fluid cells. *Pediatr Res* 1985; **19**: 334.
58. Gagne R, Lescault A, Grenier A *et al.* Prenatal diagnosis of hereditary tyrosinemia: measurement of succinylacetone in amniotic fluid. *Prenat Diagn* 1982; **2**: 185.
59. Jakobs C, Dorland L, Wikkerink B *et al.* Stable isotope dilution analysis of succinylacetone using electron capture negative ion mass fragmentography: an accurate approach to the pre- and neonatal diagnosis of hereditary tyrosinemia type I. *Clin Chim Acta* 1988; **171**: 223.
60. Grenier A, Cederbaum S, Laberge C *et al.* A case of tyrosinaemia type I with normal level of succinylacetone in the amniotic fluid. *Prenat Diagn* 1996; **16**: 239.
61. Labelle Y, Phaneuf D, Leclerc B, Tanguay RM. Characterization of the human fumarylacetoacetate hydrolase gene and identification of a missense mutation abolishing enzymatic activity. *Hum Mol Genet* 1993; **2**: 941.
62. Demers SI, Phaneuf D, Tanguay RM. Strong association of hereditary tyrosinemia type 1 with haplotype 6 in French-Canadians. Carrier detection and prenatal diagnosis by RFLP analysis. *Am J Hum Genet* 1994; **55**: 327.
63. St-Louis M, Poudrier J, Phaneuf D *et al.* Two novel mutations involved in hereditary tyrosinemia type I. *Hum Mol Genet* 1995; **4**: 319.
64. Grompe M, al-Dhalimy M. Mutations of the fumarylacetoacetate hydrolase gene in four patients with tyrosinemia type I. *Hum Mutat* 1993; **2**: 85.
65. Arranz JA, Pinol F, Kozak L *et al.* Splicing mutations, mainly IVS6-1(G>T), account for 70% of fumarylacetoacetate hydrolase (FAH) gene alterations, including 7 novel mutations, in a survey of 29 tyrosinemia type I patients. *Hum Mutat* 2002; **20**: 180.
66. Kvittingen EA, Börresen AL, Stokke O *et al.* Deficiency of fumarylacetoacetase without hereditary tyrosinemia. *Clin Genet* 1985; **27**: 550.
67. Kogut MD, Shaw KN, Donnell GN. Tyrosinosis. *Am J Dis Child* 1967; **113**: 47.
68. Christensen E, Jacobsen BB, Gregersen N *et al.* Urinary excretion of succinylacetone and δ -aminolevulinic acid in patients with hereditary tyrosinemia. *Clin Chim Acta* 1981; **116**: 331.
69. Tschudy DP, Hess RA, Frykholm BC, Blaese RM. Immunosuppressive activity of succinylacetone. *J Lab Clin Med* 1982; **99**: 526.
70. Sassa S, Kappas A. Impairment of heme synthesis by succinylacetone: a powerful inhibitor of α -aminolevulinic acid dehydratase activity produced in tyrosinemia. *Clin Res* 1982; **30**: 551A.
71. Magera MJ, Gunawardena ND, Hahn SH *et al.* Quantitative determination of succinylacetone in dried blood spots for newborn screening of tyrosinemia type I. *Mol Genet Metab* 2006; **88**: 16.
72. Turgeon C, Magera MJ, Allard P *et al.* Combined newborn screening for succinylacetone, amino acids, and acylcarnitines in dried blood spots. *Clin Chem* 2008; **54**: 657.
73. Berger R, van Faassen H, Smith GP. Biochemical studies on the enzymatic deficiencies in hereditary tyrosinemia. *Clin Chim Acta* 1983; **134**: 129.
74. Fallstrom SP, Lindblad B, Steen G. On the renal tubular damage in hereditary tyrosinemia and on the formation of succinylacetoacetate and succinylacetone. *Acta Paediatr Scand* 1981; **70**: 315.
75. Stoner E, Starkman H, Wellner D *et al.* Biochemical studies of a patient with hereditary hepatorenal tyrosinemia: evidence of glutathione deficiency. *Pediatr Res* 1984; **18**: 1332.
76. Seltzer S, Lin M. Maleylacetone cis-trans-isomerase. Mechanism of the interaction of coenzyme glutathione and

- substrate maleylacetone in the presence and absence of enzyme. *J Am Chem Soc* 1979; **101**: 3091.
77. Kvittingen EA, Rootwelt H, Brandtzaeg P. Hereditary tyrosinemia type I. *J Clin Invest* 1993; **91**: 1816.
 78. Kvittingen EA, Rootwelt H, Berger R, Brandtzaeg P. Self-induced correction of the genetic defect in tyrosinemia type I. *J Clin Invest* 1994; **94**: 1657.
 79. Blikrud YT, Brodtkorb E, Andresen PA *et al*. Tyrosinaemia type I: *de novo* mutation in liver tissue suppressing an inborn splicing defect. *J Mol Med* 2005; **83**: 406.
 80. Holme E, Lindstedt S. Tyrosinemia type I and NTBC (2-nitro-4-trifluoromethylbenzoyl-1,3-cyclohexanedione). *J Inher Metab Dis* 1998; **21**: 507.
 81. Halvorsen S. Dietary treatment of tyrosinosis. *Am J Dis Child* 1967; **113**: 38.
 82. Shasteen W, Zetterstrom R. Dietary treatment in tyrosinemia (tyrosinosis). *Am J Dis Child* 1967; **113**: 31.
 83. Halvorsen S, Gjessing LR. Studies on tyrosinosis: 1 effect of low-tyrosine and low-phenylalanine diet. *Br Med J* 1964; **2**: 1171.
 84. Halvorsen S, Kvittingen E-A, Flatmark A. Outcome of therapy of hereditary tyrosinemia. *Acta Paediatr Jpn* 1988; **30**: 425.
 85. Fisch RO, McCabe ERB, Doeden D *et al*. Homotransplantation of the liver in a patient with hepatoma and hereditary tyrosinemia. *J Pediatr* 1978; **93**: 592.
 86. Starzl TE, Zitelli BJ, Shaw BW *et al*. Changing concepts: liver replacement for hereditary tyrosinemia and hepatoma. *J Pediatr* 1985; **106**: 604.
 87. Paradis K, Weber A, Seidman EG *et al*. Liver transplantation for hereditary tyrosinemia: the Quebec experience. *Am J Hum Genet* 1990; **47**: 338.
 88. Freese DK, Tuchman M, Schwarzenberg SJ *et al*. Early liver transplantation is indicated for tyrosinemia type I. *J Pediatr Gastroenterol Nutr* 1991; **13**: 10.
 89. Luks FI, St-Vil D, Hancock BJ *et al*. Surgical and metabolic aspects of liver transplantation for tyrosinemia. *Transplantation* 1993; **56**: 1376.
 90. Tuchman M, Freese DK, Sharp HL *et al*. Contribution of extrahepatic tissues to biochemical abnormalities in hereditary tyrosinemia type I: study of three patients after liver transplantation. *J Pediatr* 1987; **110**: 399.
 91. Tazawa Y, Kikuchi M, Kurobane I *et al*. An acute form of tyrosinemia type I with multiple intrahepatic mass lesions. *J Pediatr Gastroenterol Nutr* 1990; **10**: 536.
 92. Shteyer E, Simanovsky N, Koplewitz B *et al*. Multiple hepatic lesions in a girl with tyrosinemia: not always hepatocellular carcinoma. *J Pediatr* 2011; **158**: 513.
 93. Shoemaker LR, Strife CF, Balistreri WF, Ryckman FC. Rapid improvement in the renal tubular dysfunction associated with tyrosinemia following hepatic replacement. *Pediatrics* 1992; **89**: 251.
 94. Lindstedt S, Holme E, Lock EA *et al*. Treatment of hereditary tyrosinemia type I by inhibition of 4-hydroxyl-phenylpyruvate dioxygenase. *Lancet* 1992; **340**: 813.
 95. Gibbs TC, Payan J, Brett EM *et al*. Peripheral neuropathy as the presenting feature of tyrosinemia type I and effectively treated with an inhibitor of 4-hydroxyl-phenylpyruvate dioxygenase. *J Neurol Neurosurg Psychiatr* 1993; **56**: 1129.
 96. Holme E. Presentation. 3rd Swedish Orphan Conference. Karlskoga, Sweden, May 15, 2003.

Nonketotic hyperglycinemia

Introduction	180	Treatment	185
Clinical abnormalities	181	References	185
Genetics and pathogenesis	183		

MAJOR PHENOTYPIC EXPRESSION

Potentially lethal neonatal illness, absent or poor mental development, convulsions, myoclonus, hiccups, hypotonia progressive to spasticity, abnormal electroencephalogram (EEG), hyperglycinemia, hyperglycinuria, elevated cerebrospinal fluids; plasma glycine ratio, and defective activity of the glycine cleavage system.

INTRODUCTION

Nonketotic hyperglycinemia (glycine encephalopathy) is an inborn error of amino acid metabolism in which large amounts of glycine accumulate in body fluids, and there is no demonstrable accumulation of organic acids. A majority of patients has the classic phenotype in which life-threatening illness begins in the early days of life, and most patients die if not maintained by the use of mechanical ventilation. Survivors usually display little cognitive development and often have virtually continuous seizures. The disease was first described by Gerritsen and colleagues in 1965 [1]. It was called ‘nonketotic hyperglycinemia’ [2, 3] to distinguish it from other disorders, such as propionic acidemia (Chapter 2), in which hyperglycinemia occurs.

The high concentration of glycine in the cerebrospinal fluid (CSF), and the ratio of its concentration to that of the plasma, provide the usual method of diagnosis. Analysis of organic acids of the urine is useful to exclude organic acidemia. Enzyme analysis is not generally available; the enzyme is fully expressed only in liver and brain.

The molecular defect is in the glycine cleavage system (EC 2.1.2.1.0) (Figure 22.1), which is a multienzyme complex with four protein components [4]. These have been labeled the P protein (glycine decarboxylase, GLDC), T protein (aminomethyl transferase, AMT), H protein (the lipoic acid containing protein), and L protein (a lipoamide dehydrogenase). In patients with nonketotic hyperglycinemia in whom the individual components have been studied, the majority has had defects in the P

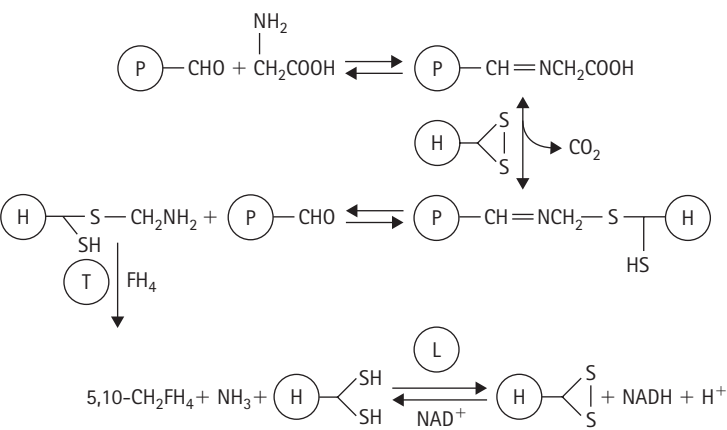


Figure 22.1 The glycine cleavage system. The protein components, circled, are labeled, P, H, T and L. (Reproduced with permission from Nyhan WL. *The Metabolic Basis of Inherited Disease*, 5th edn. Stanbury JB, Wyngaarden JB, Fredrickson DS et al. (eds). New York: McGraw Hill, 1982: 564.)

protein. Defective activity of the H and T protein has been described. The cDNA for the P protein has been cloned and a number of mutations has been identified [5, 6]. The genes for the T and H proteins have also been identified and mutations have been identified [7, 8]. More than 50 mutations in the gene for the P protein have been defined and more than 30 in the *AMT* gene. Only one patient has been documented to have defective H protein [9, 10].

CLINICAL ABNORMALITIES

In the classic phenotype, the infant appears normal at birth and there is a hiatus, usually up to 48 hours but ranging from a few hours to 8 days, in which the patient remains well. Then, usually after the initiation of protein-containing feedings, lethargy develops, along with anorexia and failure to feed or later to suck. Feeding by nasogastric tube may be initiated. There may be some vomiting, but this is usually not a prominent feature. Lethargy is progressive to coma, and within 24–48 hours of the first symptom the patient is flaccid, completely unresponsive to stimuli, and apneic (Figure 22.2) [1–3, 11–18]. A majority of patients probably die at this point. Some are ventilated artificially using a respirator for long enough to permit the diagnosis. Subsequent treatment with exchange transfusion, peritoneal dialysis, or sodium benzoate may lead to the initiation of spontaneous respirations and the discontinuation of the respirator. However, there is seldom



Figure 22.2 JS: A 4-day-old patient with nonketotic hyperglycinemia. Following exchange transfusion, assisted ventilation could be discontinued, but the patient was still in the intensive care unit and unresponsive. Illustrated is the extreme hypotonicity.

much evidence of cerebral development and most patients die within the first year of life. The disorder is diagnosed in increasing fashion in neonatal intensive care units of major medical centers, but it is likely that as many or more die neonatal deaths without benefit of diagnosis.

In the infant, the cry may be high-pitched. Suck, grasp, and Moro responses are poor. Edema has been observed rarely [11, 17]. Seizures may be myoclonic or grand mal [15]. They are prominent in almost all patients and may be virtually continuous [1, 17, 19]. Hiccupping is common and often persistent [11] and, with some frequency, we have obtained historical evidence of recurrent prenatal hiccupping. Intermittent ophthalmoplegia or wandering eye movements have been described [20]. The EEG is usually diffusely abnormal [21–25]. The typical pattern of burst-suppression is one of periodic or pseudoperiodic areas of large-amplitude sharp waves on a low voltage background [21–23]. The burst-suppression pattern has been observed as early as 30 minutes after birth [23]. This pattern, typical of the neonate, may change to hypsarrhythmia in later infancy. There may be multifocal epileptiform discharges [24]. Brainstem auditory evoked potentials may be abnormal [24].

Patients surviving the acute neonatal crisis develop a pattern of cerebral palsy with spasticity and hypertonia [1, 3], although they may be hypotonic throughout infancy [3, 11]. Deep tendon reflexes are exaggerated and there is ankle clonus. A position of opisthotonos is common. The patient may be completely unaware of surroundings and have few spontaneous movements. There is no head control or other evidence of psychomotor development, such as sitting or rolling over, and no adaptive or social behavior (Figures 22.3 and 22.4). Eye movements may be disconjugate. Gavage or gastrostomy feeding may be required.

Nonketotic hyperglycinemia is heterogeneous and, while the majority of patients display the classic phenotype, a small number has been reported in whom a variety of milder forms have been observed. At the extreme from the classic, three affected girls [26] had only mild impairment;



Figure 22.3 DG: An eight-month-old boy with nonketotic hyperglycinemia, in the tonic neck posture.



Figure 22.4 MC: An almost two-year-old with nonketotic hyperglycinemia. He survived a neonatal requirement for assisted ventilation following treatment with sodium benzoate but had little development or awareness of his environment. A feeding tube was required for nutrition. Electroencephalogram revealed almost continuous seizure activity.

only one of the three was in an institution. Other families have been reported in which there was mild developmental delay [27–32]. Acute febrile illness has been associated with involuntary movements, paresis of upward gaze, and delirium. Severe mental impairment and seizures may be found despite an atypical late onset [32]. It should be emphasized that milder variants are the exception. Of 30 patients studied by the Sendai group [5, 33], 26 (87 percent) were of the classic neonatal type and the four survivors had severely impaired mental development. The natural history of the disease was recorded through parent questionnaire in 65 patients [34]. More girls than boys died in the newborn period; mean age of female death was less than one month, while that of boys was 2.6 years. Ten boys were able to walk.

We have encountered a very different presentation [35] as a neurodegenerative disease not unlike Tay-Sachs or



Figure 22.5 LS: At ten months. This patient presented with the picture of a cerebral degenerative disorder.

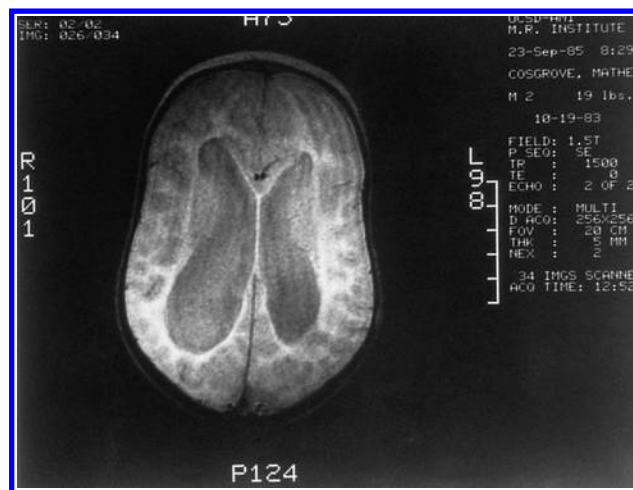


Figure 22.6 Magnetic resonance image of the brain of the patient in Figure 22.3. Dilated ventricles and sulci indicated a severe loss of volume of brain.

Krabbe disease. The patient developed relatively normally for the first months of life and then in the second half of the first year showed progressive cerebral deterioration. This led to a state of decerebrate rigidity (Figure 22.5) followed by death.

Magnetic resonance imaging (MRI) of the brain (Figure 22.6) [36–38], or computed tomography (CT), in this disease shows progressive atrophy and delayed myelination. The corpus callosum was abnormally thin in all patients and volume loss was both supra- and infratentorial. T₂-weighted images revealed decreased or absent myelination in the supratentorial white. These observations are consistent with reported neuropathology, including atrophy and corpus callosal thinning. Spongy rarefaction and vacuolation of myelin (Figure 22.7), as well as variable gliosis have been observed regularly [39, 40].

Transient nonketotic hyperglycinemia represents a clinical presentation indistinguishable from the classic

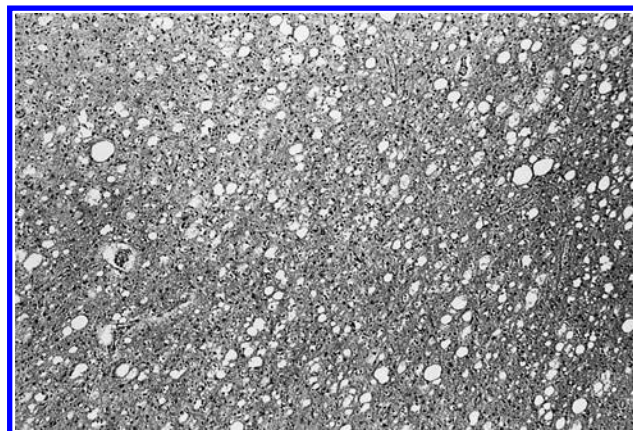


Figure 22.7 Histological section of the cortical white matter indicating the typical neuropathological finding of spongy degeneration and gliosis.

neonatal nonketotic hyperglycinemia [41]. The EEG may display the burst-suppression pattern, and the concentrations of glycine in plasma and CSF, and the CSF to plasma ratios may be diagnostic. Surprisingly, by 2–8 weeks of age, glycine levels have returned to normal. Normal cognitive and neurologic function at follow up has been reported. At least 12 patients have been reported, but with the advent of molecular diagnosis, many of these patients have been shown to have mutations in the GLDC genes.

This syndrome was thought to be a consequence of immaturity of the enzyme system; support for the idea came from studies of the development of the glycine cleavage system in the neonatal rat [42]. The existence of the syndrome has created an ethical dilemma for physicians and families of an infant in coma being artificially ventilated once a diagnosis of nonketotic hyperglycinemia has been made. Many families, once the diagnosis and its bleak prognosis have been understood, have elected to discontinue life support. The alternatives seemed to be either death despite all support or, once the critical period has passed, no longer need for ventilator support, but no development either. The existence of the transient syndrome raised the possibility of a third option. Most of us have never seen such a patient. It seems likely that the disease is not transient, but the symptomatology and bad prognosis may be leaving the ethical issues unresolved. However, it is clear that these patients are a small minority.

GENETICS AND PATHOGENESIS

Deficiency of any of the components of the glycine cleavage is transmitted in autosomal recessive fashion [33]. Defective activity of overall glycine cleavage was first described *in vivo* in studies of the metabolism of ^{14}C -labeled glycine [2]. Patients displayed virtually no conversion of glycine-1- ^{14}C to respiratory $^{14}\text{CO}_2$ and the conversion of glycine-2- ^{14}C to the third carbon of serine was similarly defective. Assay of the enzyme in the liver homogenates was reported in 1969 by Tada and colleagues [43]. The enzyme system is expressed in liver, kidney, and brain, and until recently could only be demonstrated in those tissues. Recently, it has been shown that the system is induced in the transformation of B lymphocytes with Epstein–Barr virus [44, 45]. This has turned out to be a convenient, but unreliable, approach to the diagnosis.

The glycine cleavage system (Figure 22.1) is a mitochondrial complex with four individual protein components [4, 26, 46]. The P protein is a pyridoxal phosphate-dependent glycine decarboxylase. The P and H proteins, lipoic acid-containing proteins, are required for the formation of CO_2 from glycine. All four are required for the conversion of glycine to CO_2 , NH_3 and a one-carbon tetrahydrofolate (FH_4) derivative, which can then function in one carbon transfer, as in the formation of serine from glycine. The T protein contains FH_4 and the L protein is a lipoamide dehydrogenase.

Analysis of hepatic activity of the glycine cleavage system in 30 patients in Sendai [5, 33, 47] revealed undetectable levels in the classic disease and some residual activity in more indolent patients. Analysis of the protein component of the complex revealed that 87 percent had abnormalities in the P protein, and this included all of the classic patients. Four patients had defects in the T protein. In seven patients in whom the brain enzyme was assayed, the same component as in liver was found to be defective. Of atypical patients, two had defects in T protein and one in H protein [33, 48]. The patient with the cerebral degenerative phenotype had defective activity of both the P and H proteins [49], but the content of the P protein was normal, and it was concluded that the H protein was structurally abnormal. Immunochemical studies in patients with the classic presentation revealed virtually no P protein [50].

Molecular studies of mutation have revealed heterogeneity for the gene of the P protein, and mutations of the T and H protein. The P protein gene [5, 6] has been located on chromosome 9p13-23 [51], as first suggested by a patient with nonketotic hyperglycinemia and the 9p- syndrome [52]. It contains 25 exons over 135 kb. The gene for the H protein codes for a precursor protein of 173 amino acids and a mature protein of 125 amino acids [53]. The T protein gene maps to chromosome 3p21.2-21.1 [54]. It has 6 kb over nine exons. The H protein was mapped to chromosome 16q24 [55]. It spans 13.5 kb and contains five exons.

In a Japanese patient, a three-base deletion was found in the P protein gene, which led to deletion of a phenylalanine at position 756 [6]. Expression of the normal and mutant protein in Cos 7 cells led to abundant P protein activity in cells with the normal gene and no activity in those with the mutant gene.

Nonketotic hyperglycinemia is common in northern Finland where it occurs in one of 12,000 births [56]. This severe form of the disease was found to result from a point mutation of a G to T at nucleotide 1691 resulting in a change from serine to leucine at residue number 564 of the P protein [57]. The absence of this mutation in 20 non-Finnish alleles and its presence in ten unrelated Finnish patients indicates the presence of a founder effect. Both this mutation and the Japanese deletion occurred in a region of the protein that is thought to be important for enzyme activity and for the binding of pyridoxal phosphate (Figure 22.8). In contrast, a patient with late onset nonketotic hyperglycinemia had a methionine to isoleucine change at position 391 in a very different part of the molecule [5].

In a comprehensive screening for mutations, Kure *et al.* [58] identified P or T protein mutations in 75 percent of neonatal and 83 percent of infantile families. No H protein mutations were found. In 16 of 36 families, mutations were found on only one allele. Seven missense mutations were clustered in exon 19 at the cofactor binding site Lys754; a large deletion in exon 1 was found in Caucasian, Asian, and Black families with multiple origins indicated by haplotype analysis.

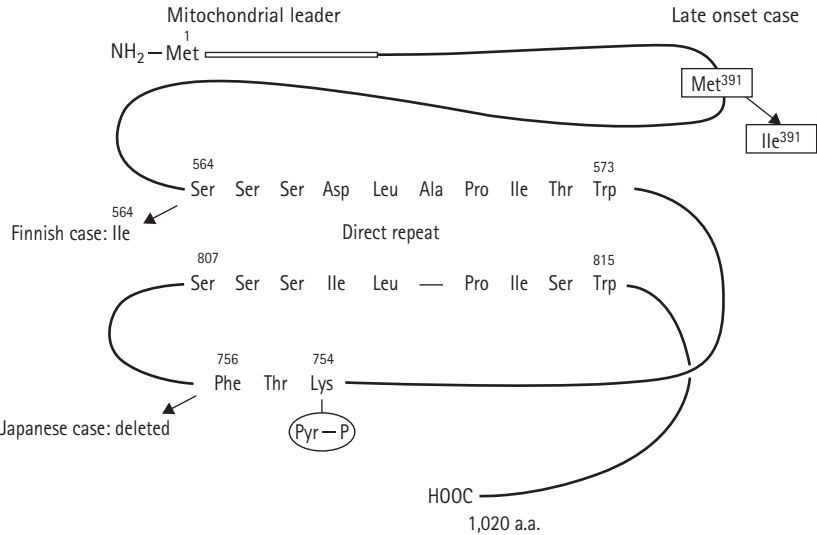


Figure 22.8 Molecular alterations in three different forms of the P protein of the glycine cleavage enzyme. The Japanese and Finnish phenotypes were of the classic type, while the patient with the methionine to isoleucine change at 391 had a more indolent disease. (Reprinted with kind permission from Springer Science & Business Media [5].)

Mutations in the gene for the T protein have included a G to A transition coding for a glycine to aspartic acid substitution at 269 (G269D) in a patient with classic neonatal disease [7], an A to G change leading to a histidine to arginine substitution at 42 (H42R) in an Israeli-Arab family [59], a deletion (183delC) causing a frameshift, and a G to C change causing D276H [8]. An atypical patient was a compound for two G to A changes, G47R and R320H. A novel splice-site mutation was found in three unrelated families [60].

Conventional approaches to genetic assistance to families are difficult in disorders in which the enzyme does not express in fibroblasts or amniocytes. Prenatal diagnosis of this disease became possible with the recognition that the cleavage system did express in chorionic villus samples. Thirty-one pregnancies were monitored [5], of which 23 were normal and eight affected. Similar results have been reported in experience with 50 pregnancies at risk [61], but 10 percent residual activity may make prenatal diagnosis inaccurate. Enzyme assay of cultured lymphoblasts has given intermediate results for heterozygote detection, but this approach may be inaccurate. Identification of the mutation permits prenatal diagnosis and detection of heterozygotes using molecular biology, and a prenatal diagnosis of the Finnish mutation has been made [57].

Concentrations of glycine are elevated in the blood, urine, and CSF. In spite of the large amounts of glycine found in the urine, it is possible to miss a patient with hyperglycinemia when screening the urine for amino acids by paper chromatography or electrophoresis. The normal glycine spot is very prominent. Also, patients are often studied when acutely ill, not eating, and being maintained on parenterally administered fluids. Under these circumstances, the excretion of glycine in hyperglycinemic patients may be normal. In general, it is better to screen for hyperglycinemia by assaying blood rather than urine. Blood concentrations are seldom brought into the normal range.

Table 22.1 Ratios of the concentration of glycine in the cerebrospinal fluid to that of plasma

Subject	Ratio
Finnish mean [64]	0.11
NKH, RH [11]	0.30
NKH, TZ [3]	0.10
Neurodegenerative variant [35]	0.07
Milder variant [29]	0.07
Control mean [62]	0.02

The concentrations of glycine are uniquely elevated in the CSF. Concentrations in reported patients have varied from 130 to 360 mmol/L [11, 62, 63]. In a series of 12 patients summarized from the literature, the mean value was 93 mmol/L [27]. In the Finnish series of 19 patients, the mean was 93 mmol/L [64]. In control subjects, the concentration has generally been less than 13 mmol/L. The ratio of the CSF concentration to that of the plasma is substantially higher in patients with nonketotic hyperglycinemia than in hyperglycinemic patients with organic acidemia. In the series of 12 patients from the literature, the mean ratio was 0.17 ± 0.09 , and in the Finnish series the mean was 0.11, whereas in control individuals the ratio was 0.02 (Table 22.1). It is important to recognize that the ratio may be meaningless if concentrations are normal. A diagnosis of hyperglycinemia requires an elevated level of glycine in plasma.

We have observed patients with milder degrees of clinical expression in whom the ratios, though abnormal, were less elevated than in the classic phenotype.

Glycine has long been known to be active in the nervous system, but older information on glycine as an inhibitory neurotransmitter at strychnine receptors never fitted with the picture of intractable seizures [65]. It is now clear that glycine is an excitatory neurotransmitter

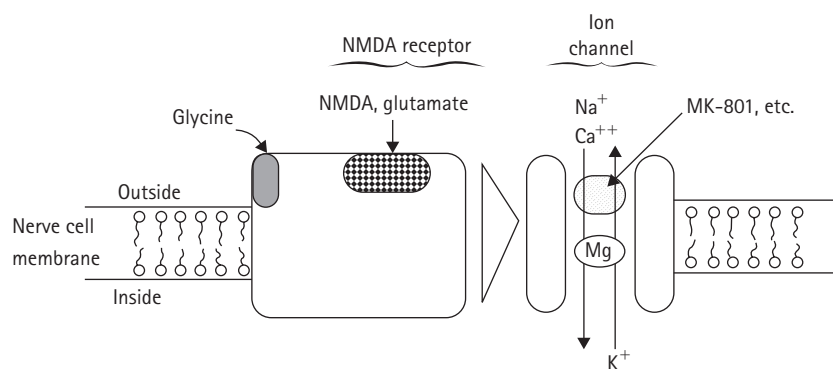


Figure 22.9 The N-methyl-D-aspartate receptor and the role of glycine. When glutamate or NMDA binds to the receptor the channel opens and positively charged ions flow to the nerve cell. Glycine binds at a different site on the receptor and acts as a facilitator.

(Figure 22.9) with a profound influence on the N-methyl-D-aspartate (NMDA) receptor, which normally responds to the excitatory amino acid glutamate [66, 67]. Glycine functions as an allosteric agonist permitting glutamate to be excitatory at much smaller contractions.

TREATMENT

The management of this disease is considerably less than satisfactory. Exchange transfusion or dialysis, or sodium benzoate, may be life-saving in the neonatal period and may permit weaning from the ventilator, but many families made aware of the grim prognosis prefer not to take these steps. The plasma concentrations of glycine may be lowered by dietary restriction or by the administration of sodium benzoate. It is now clear that treatment with large amounts of benzoate, which joins with glycine to form hippurate which is then excreted in the urine, can actually lower CSF concentration of glycine, and that there are dose–response relationships [68]. Patients so treated had a substantial decrease in seizures. Doses employed have ranged from 250 to 700 mg/kg per day. Developmental progress has been disappointing, except in one patient who was reported as developmentally normal [69].

We and others [70] have added dextromethorphan to the benzoate regimen as a noncompetitive antagonist at the NMDA receptor. Dosage employed has been in the range of 5–22 mg/kg per day. Among the four patients reported from Baltimore [70, 71], very beneficial effects on development were observed in a patient treated from the first days of his life. This was less evident in our experience, and results were mixed in the other Baltimore patients, including one who died at 12 weeks. Similarly mixed results were reported by others [69, 72]. Arnold *et al.* [73] pointed out that dextromethorphan is metabolized very differently in different individuals and that measuring levels is necessary to ensure adequate dosage. Anticonvulsant effects were associated with levels of 50–100 ng/mL. Cimetidine, an inhibitor of P450 activity, was found to increase dextromethorphan levels in a rapid metabolizing individual.

Benzoate treatment has been observed to lead to carnitine deficiency in patients with this disease [73, 74].

It would appear prudent to supplement patients under treatment with carnitine and to measure levels. It is also prudent to avoid treatment with valproate in this disease, because it causes increase in levels of glycine [75] and inhibits the activity of the glycine cleavage system [76].

REFERENCES

- Gerritsen T, Kaveggia E, Waisman HA. A new type of idiopathic hyperglycinemia with hypo-oxaluria. *Pediatrics* 1965; **36**: 882.
- Ando T, Nyhan WL, Gerritsen T *et al.* Metabolism of glycine in the nonketotic form of hyperglycinemia. *Pediatr Res* 1968; **2**: 254.
- Ziter FA, Bray PF, Madsen JA, Nyhan WL. The clinical findings in a patient with nonketotic hyperglycinemia. *Pediatr Res* 1968; **2**: 250.
- Kikuchi G. The glycine cleavage system: composition reaction mechanism and physiological significance. *Mol Cell Biochem* 1973; **1**: 169.
- Tada K, Kure S. Nonketotic hyperglycinaemia: molecular lesion diagnosis and pathophysiology. *J Inherit Metab Dis* 1993; **16**: 691.
- Kure S, Narisawa K, Tada K. Structural and expression analyses of normal and mutant mRNA encoding glycine decarboxylase: three base deletion in mRNA causes nonketotic hyperglycinemia. *Biochem Biophys Res Commun* 1991; **174**: 1176.
- Nanao K, Okamura-Ikeda K, Motokawa Y *et al.* Identification of the mutations in the T-protein gene causing typical and atypical nonketotic hyperglycinemia. *Hum Genet* 1994; **93**: 655.
- Kure S, Shinka T, Sakata Y *et al.* A one base deletion (183delC) and a missense mutation (D276H) in the T protein gene from a Japanese family with nonketotic hyperglycinemia. *J Hum Genet* 1998; **43**: 135.
- Applegarth DA, Toone JR. Glycine encephalopathy (nonketotic hyperglycinaemia): review and update. *J Inherit Metab Dis* 2004; **27**: 417.
- Kure S, Kojima K, Ichinohe A *et al.* A comprehensive mutation analysis of GLDC, AMT, and GCSH in glycine encephalopathy. *J Inherit Metab Dis* 2003; **26**: 66.
- Baumgartner R, Ando T, Nyhan WL. Nonketotic hyperglycinemia. *J Pediatr* 1969; **75**: 1022.

12. Simila S, Visakorpi JK. Clinical findings in three patients with nonketotic hyperglycinaemia. *Ann Clin Res* 1970; **2**: 151.
13. Ferdinand W, Gordon RR, Owen G. Nonketotic hyperglycinaemia: clinical findings and amino acid analyses on the plasma of a new case. *Clin Chim Acta* 1970; **30**: 745.
14. Bachmann C, Mihatsch MJ, Baumgartner RE *et al*. Nicht-Ketotische Hyperglyzinämie: Perakuter verlauf im Neugeborenenalter. *Helv Padiatr Acta* 1971; **26**: 228.
15. Von Wendt L, Simila S, Hirvasniemi A, Suvanto E. Nonketotic hyperglycinemia. A clinical analysis of 19 Finnish patients. *Monogr Hum Genet* 1978; **9**: 58.
16. DeGroot CJ, Hommes FA, Touwen BCL. The altered toxicity of glycine in nonketotic hyperglycinemia. *Hum Hered* 1977; **27**: 178.
17. Holmqvist P, Polberger S. Neonatal nonketotic hyperglycinemia (NKH). Diagnoses and management in two cases. *Neuropediatrics* 1985; **16**: 191.
18. Dalla Bernardina B, Aicardi J, Goutieres F, Plouin P. Glycine encephalopathy. *Neuropediatrics* 1979; **10**: 209.
19. Mignone F, Balbo L, Valpreda A *et al*. Iperglicinemia non chetotica. Presentazione di un caso. *Minerva Pediatr* 1980; **32**: 111.
20. Macdonald JT, Sher PK. Ophthalmoplegia as a sign of metabolic disease in the newborn. *Neurology* 1977; **27**: 971.
21. Aicardi J, Goutieres F. Encephalopathie myoclonique neonatale. *Rev Electroencephalogr Neurophysiol* 1978; **8**: 99.
22. Mises J, Moussalli-Salefranque F, Plouin P *et al*. L'EEG dans les hyperglycinemies sans cetose. *Rev Electroencephalogr Neurophysiol* 1978; **8**: 102.
23. Von Wendt L, Simila S, Saukkonen A-L *et al*. Prenatal brain damage in nonketotic hyperglycinemia. *Am J Dis Child* 1981; **135**: 1072.
24. Markand ON, Bhuwan PG, Brandt IK. Nonketotic hyperglycinemia: electroencephalographic and evoked potential abnormalities. *Neurology* 1982; **32**: 151.
25. Bernardina BD, Dulac O, Fejerman H *et al*. Early myoclonic epileptic encephalopathy (EMEE). *Eur J Pediatr* 1983; **140**: 248.
26. Ando T, Nyhan WL, Bicknell WL *et al*. Nonketotic hyperglycinaemia in a family with an unusual phenotype. *J Inherit Metab Dis* 1978; **1**: 79.
27. Holmgren G, Blomquist HK. Nonketotic hyperglycinemia in 2 sibs with mild psycho-neurological symptoms. *Neuropediatrics* 1977; **8**: 67.
28. Flannery DB, Pellock J, Bousounis D *et al*. Nonketotic hyperglycinemia in two retarded adults: a mild form of infantile nonketotic hyperglycinemia. *Neurology* 1983; **33**: 1064.
29. Frazier DM, Summer GK, Chamberlin HR. Hyperglycinuria and hyperglycinemia in two siblings with mild developmental delays. *Am J Dis Child* 1978; **132**: 777.
30. Nightingale S, Barton ME. Intermittent vertical supranuclear ophthalmoplegia and ataxia. *Mov Disord* 1991; **6**: 76.
31. Steiner RD, Sweetser DA, Rohrbach JR *et al*. Nonketotic hyperglycinemia: atypical clinical and biochemical manifestations. *J Pediatr* 1996; **128**: 243.
32. Singer HS, Valle D, Hayasaka K, Tada K. Nonketotic hyperglycinemia: studies in an atypical variant. *Neurology* 1989; **39**: 286.
33. Tada K. Nonketotic hyperglycinemia: clinical and metabolic aspects. *Enzyme* 1987; **38**: 27.
34. Hoover-Fong JE, Shah S, Van Hove JLK. Natural history of glycine encephalopathy in 65 patients. *J Inherit Metab Dis* 2003; **26**: 64.
35. Trauner DA, Page T, Greco C *et al*. Progressive neurodegenerative disorder in a patient with nonketotic hyperglycinemia. *J Pediatr* 1981; **98**: 272.
36. Press GA, Barshop BA, Haas RH *et al*. Abnormalities of the brain in nonketotic hyperglycinemia: MR manifestation. *Am J Neuroradiol* 1989; **10**: 315.
37. Dobyns WB. Agenesis of the corpus callosum and gyral malformations are frequent manifestations of nonketotic hyperglycinemia. *Neurology* 1989; **39**: 817.
38. Rogers T, Al-Rayess M, O'Shea P, Ambler MW. Dysplasia of the corpus callosum in identical twins with nonketotic hyperglycinemia. *Pediatr Pathol* 1991; **11**: 897.
39. Shuman RM, Leech RW, Scott CR. The neuropathology of the nonketonic and ketonic hyperglycinemias: three cases. *Neurology* 1978; **28**: 139.
40. Brun A, Borjeson M, Hultberg B *et al*. Nonketotic hyperglycinemia: a clinical biochemical and neuropathologic study including electronic microscopy findings. *Neuropediatrics* 1979; **10**: 195.
41. Luder AS, Davidson A, Goodman SI, Greene CL. Transient nonketotic hyperglycinemia in neonates. *J Pediatr* 1989; **114**: 1013.
42. Kalbag SS, Palekar AG. Postnatal development of the glycine cleavage system in rat liver. *Biochem Med Metab Biol* 1990; **43**: 128.
43. Tada K, Narisawa K, Yoshida T *et al*. Hyperglycinemia: a defect in glycine cleavage reaction. *Tohoku J Exp Med* 1969; **98**: 289.
44. Kure S, Narisawa K, Tada K. Enzymatic diagnosis of nonketotic hyperglycinemia with lymphoblasts. *J Pediatr* 1992; **120**: 95.
45. Christodoulou J, Kure S, Hayasaka K, Clarke JTR. Atypical nonketotic hyperglycinemia confirmed by assay of the glycine cleavage system in lymphoblasts. *J Pediatr* 1993; **123**: 100.
46. Motokawa Y, Kikuchi G. Glycine metabolism by rat liver mitochondria. Reconstitution of the reversible glycine cleavage system with partially purified protein components. *Arch Biochem Biophys* 1974; **164**: 624.
47. Hayasaka K, Tada K, Kikuschi G *et al*. Nonketotic hyperglycinemia: two patients with primary defects of P-protein and T-protein respectively in the glycine cleavage system. *Pediatr Res* 1983; **17**: 926.
48. Tada K, Hayasaka K. Clinical and biochemical aspects. *Eur J Pediatr* 1987; **146**: 221.
49. Hiraga K, Kochi H, Hayasaka K *et al*. Defective glycine cleavage system in nonketotic hyperglycinemia. *J Clin Invest* 1981; **68**: 525.
50. Hayasaka K, Tada K, Nyhan WL *et al*. Nonketotic hyperglycinemia: analyses of the glycine cleavage system in typical and atypical cases. *J Pediatr* 1987; **110**: 873.
51. Tada K, Kure S. Nonketotic hyperglycinemia: molecular lesion and pathophysiology. *Int Pediatr* 1993; **8**: 52.
52. Burton BK, Pettenati MJ, Block SM *et al*. Nonketotic

- hyperglycinemia in a patient with the 9p- syndrome. *Am J Med Genet* 1989; **32**: 504.
53. Koyata H, Hiraga K. The glycine cleavage system: structure of a cDNA encoding human H-protein and partial characterization of its gene in patients with hyperglycinemias. *Am J Hum Genet* 1991; **48**: 351.
54. Nanao K, Takada G, Takahashi E *et al*. Structure and chromosomal localization of the gene encoding human T-protein of the glycine cleavage system. *Genomics* 1994; **19**: 27.
55. Kure S, Kojima K, Kudo T *et al*. Chromosomal localization, structure, single-nucleotide polymorphisms, and expression of the human H-protein gene of the glycine cleavage system (GCSH), a candidate gene for nonketotic hyperglycinemia. *J Hum Genet* 2001; **46**: 378.
56. Von Wendt L, Hirvasniemi A, Simila S. Nonketotic hyperglycinemia: a genetic study of 13 Finnish families. *Clin Genet* 1979; **15**: 411.
57. Kure S, Takayanagi M, Narisawa K *et al*. Identification of a common mutation in Finnish patients with nonketotic hyperglycinemia. *J Clin Invest* 1992; **90**: 160.
58. Kure S, Kato K, Dinopoulos A *et al*. Comprehensive mutation analysis of GLDC, AMT, and GCSH in nonketotic hyperglycinemia. *Hum Mutat* 2006; **27**: 343.
59. Kure S, Mandel H, Rolland MO *et al*. A missense mutation (His42Arg) in the T-protein gene from a large Israeli-Arab kindred with nonketotic hyperglycinemia. *Hum Genet* 1998; **102**: 430.
60. Toone JR, Applegarth DA, Coulter-Mackie MB, James ER. Identification of the first reported splice site mutation (IVS7-1G-A) in the aminomethyltransferase (T-protein) gene (AMT) of the glycine cleavage complex in 3 unrelated families with nonketotic hyperglycinemia. *Hum Mutat* 2000; **17**: 76.
61. Toone JR, Applegarth DA, Levy HL. Prenatal diagnosis of NKH: experience in 50 at-risk pregnancies. *J Inherit Metab Dis* 1994; **17**: 342.
62. Perry TL, Urquhart N, Maclean J *et al*. Nonketotic hyperglycinemia. *N Engl J Med* 1975; **292**: 1269.
63. Scriver CR, White A, Sprague W, Horwood SP. Plasma-CSF glycine ratio in normal and nonketotic hyperglycinemic subjects. *N Engl J Med* 1975; **293**: 778.
64. Von Wendt L, Simila S, Hirvasniemi A, Suvanto E. Altered levels of various amino acids in blood plasma and cerebrospinal fluid of patients with nonketotic hyperglycinemia. *Neuropadiatrie* 1978; **9**: 360.
65. Krnjevic K. Chemical nature of synaptic neurotransmission in vertebrates. *Physiol Rev* 1974; **54**: 418.
66. Newell DW, Barth A, Ricciardi TN, Malouf AT. Glycine causes receptors in the hippocampus. *Exp Neurol* 1997; **145**: 235.
67. Johnson JW, Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* 1987; **325**: 529.
68. Wolff JA, Kulovich S, Yu A *et al*. The effectiveness of benzoate in the management of seizures in nonketotic hyperglycinemia. *Am J Dis Child* 1986; **140**: 596.
69. Boneh A, Degani Y, Harari M. Prognostic clues and outcome of early treatment of nonketotic hyperglycinemia. *Pediatr Neurol* 1996; **15**: 137.
70. Hamosh A, McDonald JW, Valle D *et al*. Dextromethorphan and high-dose benzoate therapy for nonketotic hyperglycinemia in an infant. *J Pediatr* 1992; **121**: 131.
71. Hamosh A, Maher JF, Bellus GA *et al*. Long-term use of high-dose benzoate and dextromethorphan for the treatment of nonketotic hyperglycinemia. *J Pediatr* 1998; **132**: 709.
72. Zammarchi E, Kure S, Hayasaka K, Clarke JTR. Failure of early dextromethorphan and sodium benzoate therapy in an infant with nonketotic hyperglycinemia. *Neuropediatrics* 1994; **25**: 274.
73. Arnold GL, Griebel ML, Valentine JL *et al*. Dextromethorphan in nonketotic hyperglycinemia: metabolic variation confounds the dose-response relationship. *J Inherit Metab Dis* 1997; **20**: 28.
74. Van Hove JL, Kishnani P, Muenzer J *et al*. Benzoate therapy and carnitine deficiency in non-ketotic hyperglycinemia. *Am J Med Genet* 1995; **59**: 444.
75. Belkingsopp WK, DuPont PA. Dipropylacetate (valproate) and glycine metabolism. *Lancet* 1977; **2**: 617.
76. Kochi H, Hawasaka W, Hiraga K, Kikuchi G. Reduction of the level of glycine cleavage system in the rat liver resulting from administration of dipropylacetic acid: an experimental approach to hyperglycinemia. *Arch Biochem Biophys* 1979; **198**: 589.

HYPERAMMONEMIA AND DISORDERS OF THE UREA CYCLE

23.	Introduction to hyperammonemia and disorders of the urea cycle	191
24.	Ornithine transcarbamylase deficiency	197
25.	Carbamylphosphate synthetase deficiency	205
26.	Citrullinemia	210
27.	Argininosuccinic aciduria	216
28.	Argininemia	223
29.	Hyperornithinemia, hyperammonemia, homocitrullinuria syndrome	229
30.	Lysinuric protein intolerance	235
31.	Glutamine synthetase deficiency	241

Introduction to hyperammonemia and disorders of the urea cycle

Work up of the patients with hyperammonemia	192
Treatment of hyperammonemia	193

References	196
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Distinct disorders involve the enzymes of every step in the urea cycle (Figure 23.1) [1, 2]. These include ornithine transcarbamylase deficiency (Chapter 24), citrullinemia (Chapter 26), carbamyl phosphate synthetase deficiency (Chapter 25), argininosuccinic aciduria (Chapter 27), and argininemia (Chapter 28), as well as N-acetylglutamate synthetase deficiency. In addition, there is a syndrome of transient hyperammonemia of the newborn [3], in which the early clinical manifestations mimic those of the severe defects of urea cycle enzymes and may be fatal, but if the patient can get through the first 5 days of life, the problem disappears and prognosis is good.

A hyperammonemic syndrome is also characteristic of the HHH (hyperammonemia, hyperornithinemia, and homocitrullinuria) syndrome (Chapter 29), which is caused by defective transport of ornithine into the mitochondria. Lysinuric protein intolerance (Chapter 30) is also associated with episodic hyperammonemia, but its major expression is extreme failure to thrive.

Deficiencies of enzymes of the urea cycle lead to hyperammonemia, and they present classically with sudden neonatal coma and a picture of overwhelming illness. The most classic of these presentations is that of ornithine transcarbamylase deficiency in the male. This

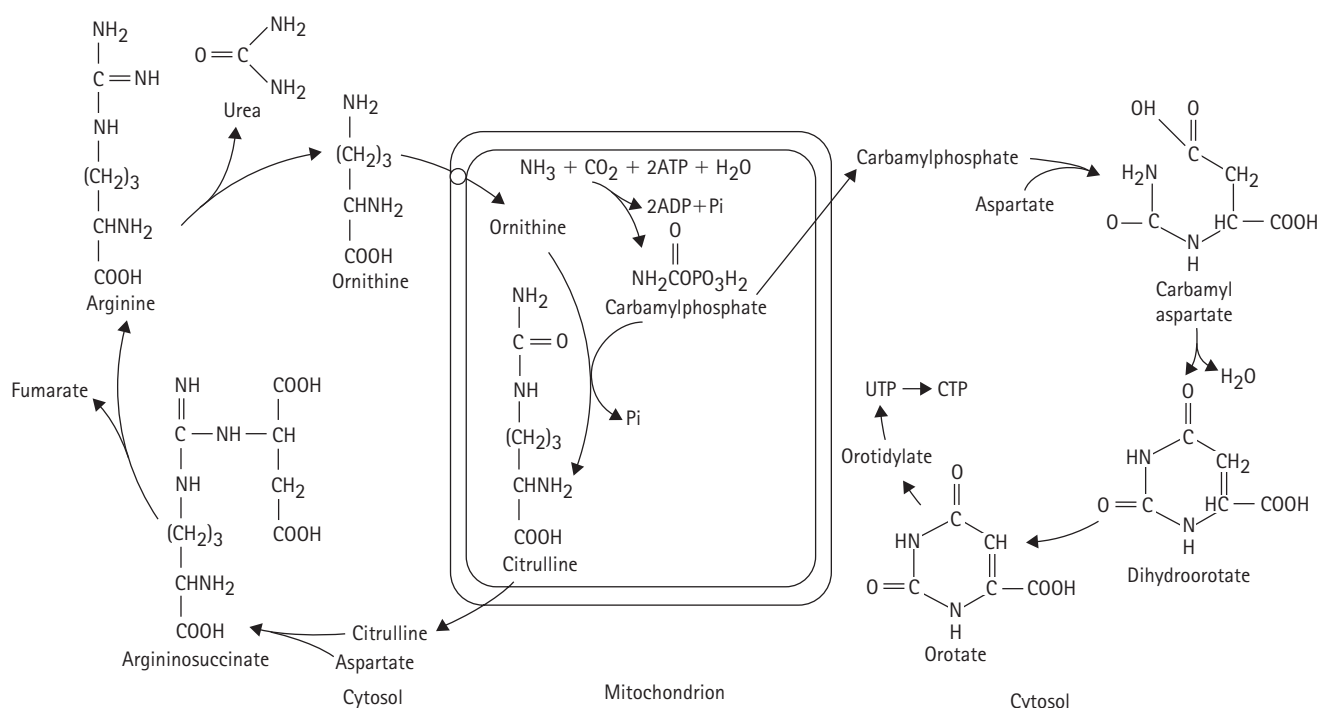


Figure 23.1 The urea cycle.

classic presentation of the hyperammonemic diseases is with acute neonatal life-threatening coma. Lethargy leads to a state of complete unresponsiveness reminiscent of surgical anesthesia. Breathing stops, and in the absence of intubation and artificial ventilation, death ensues. This clinical picture and concentration of ammonia from 400 to 2000 $\mu\text{mol/L}$ are seen in a variety of disorders in addition to those listed above. These include the organic acidemias and the disorders of fatty acid oxidation. Effective management requires a precise diagnosis, but vigorous therapy should be initiated immediately on recognition of the hyperammonemia.

WORK UP OF THE PATIENTS WITH HYPERAMMONEMIA

A systematic approach to the work up of an infant in hyperammonemic coma is shown in Figure 23.2. The differential diagnosis is important because different disorders require very different treatments. It must proceed with dispatch in order to institute appropriate therapy. The initial studies can be carried out in any clinical laboratory and provide clear direction to the next diagnostic and therapeutic steps. Ultimately, studies must be carried out in a laboratory that specializes in biochemical genetic analysis

in order to make a precise definitive diagnosis. Liver biopsy and enzymatic analysis may be required for the diagnosis of ornithine transcarbamylase deficiency, carbamyl phosphate synthetase deficiency, or N-acetylglutamate synthetase deficiency; but mutational analysis provides a less invasive approach that often, but not always, provides the definitive diagnosis. In an infant in coma, the blood concentration of ammonia should be measured. A work up for hyperammonemia should be undertaken in any newborn with an ammonia concentration greater than 150 $\mu\text{mol/L}$ and in any older infant and adult at values over 100 $\mu\text{mol/L}$. The serum concentrations of bicarbonate, sodium, and chloride are measured, the anion gap assessed, and the urine tested for ketones. The presence of acidosis and an anion gap, or massive ketosis, indicates that hyperammonemia is due to one of the organic acidemias. These disorders include propionic acidemia (Chapter 2), methylmalonic acidemia (Chapter 3), isovaleric acidemia (Chapter 7), glutaric aciduria type II (Chapter 44). The specific diagnosis is made by organic acid analysis of the urine or the acylcarnitine profile of the blood.

Hyperammonemia may also be seen in acute exacerbation of disorders of fatty acid oxidation. These episodes are characteristically hypoketotic. They are usually associated with hypoglycemia, raising the possibility of a diagnosis of Reye syndrome, but we have seen an acute

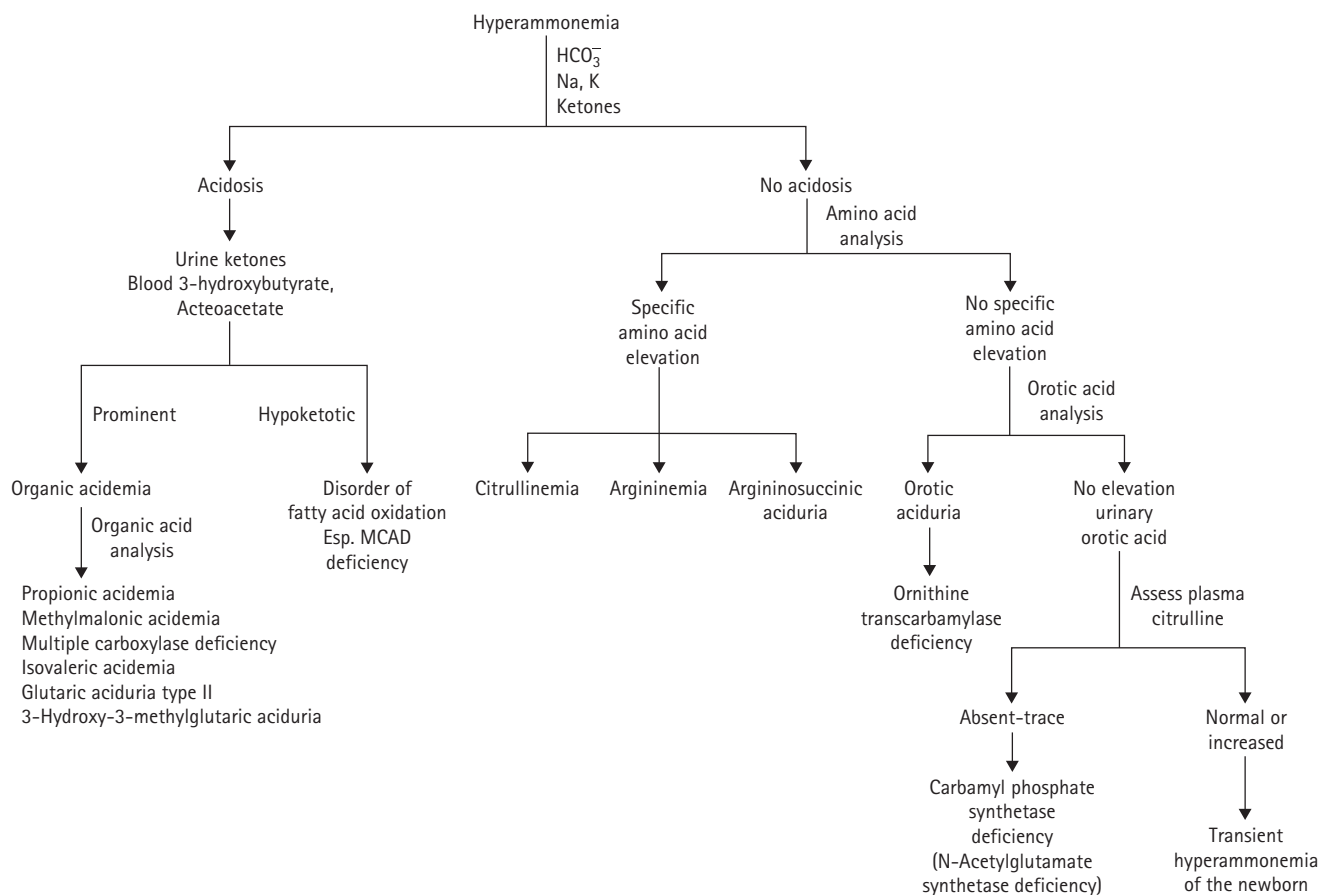


Figure 23.2 Diagnostic work up of the hyperammonemic infant in coma.

hyperammonemic episode in a teenage girl who turned out to have medium chain acyl CoA dehydrogenase deficiency (Chapter 35) that met all the criteria for a diagnosis of ornithine transcarbamylase (OTC) deficiency, except that when the liver was biopsied its OTC activity was normal [4]. Another patient with orotic acid in the urine, who had sufficient elevation of ammonia to indicate liver biopsy and enzyme assay, turned out to have uridylylmonophosphate (UMP) synthetase deficiency (Chapter 69).

Hyperammonemic patients who do not have an organic acidemia are seldom acidotic. If there is an abnormality in acid-base balance in a patient with a urea cycle defect, it is more likely to be a respiratory alkalosis, although apnea and hypoxia can lead to lactic acidosis; so adequate oxygenation and perfusion should be assured before these assessments are made once an organic acidemia is excluded. The next step toward definitive differential diagnosis is the quantitative assay of the concentrations of amino acids in blood and urine. Assessment of the plasma concentrations of amino acids will provide the diagnosis in patients with argininemia and citrullinemia. Study of the urine is required in argininosuccinic aciduria (Chapter 28).

In hyperammonemic patients found not to have diagnostic abnormality in the concentration of an amino acid, the urine should be tested for the excretion of orotic acid. Orotic aciduria is found in patients with OTC deficiency. It is also found in citrullinemia and in argininemia. In a patient without an elevation of a specific amino acid and without orotic aciduria, the expected diagnosis is carbamylphosphate synthetase (CPS) deficiency, N-acetyl-glutamate synthase deficiency or transient hyperammonemia of the newborn [3]. The diagnosis of CPS deficiency is often made by biopsy of the liver. It is preferable to make this distinction by liver biopsy after waiting, because transient hyperammonemia of the newborn resolves within 5 days. Also, this gives time to bring the patient into good metabolic control, and mutational analysis may provide the answer. The concentration of citrulline in the blood may be helpful in making these distinctions. It is usually normal or elevated in transient hyperammonemia of the newborn, whereas it is barely detectable in neonatal carbamyl phosphate synthetase or OTC deficiencies. Citrulline synthesis is coupled with the concentration of ATP. Hypocitrullinemia may be seen in disorders of the electron transport chain such as that resulting from the NARP mutation. In citrullinemia, concentrations of citrulline in plasma usually exceed 1000 $\mu\text{mol/L}$. They are elevated to levels of 50–250 $\mu\text{mol/L}$ in argininosuccinic aciduria, and to $54 \pm 22 \mu\text{mol/L}$ in transient hyperammonemia of the newborn. The normal range is 6–20 $\mu\text{mol/L}$. Symptomatic hyperammonemia may also result from a urinary tract infection in which the infecting *Proteus mirabilis* has urease activity, which produces ammonia from urea. A patient with a prune-belly syndrome and massive dilatation of the urinary tract developed coma with a blood concentration of ammonia of 140 $\mu\text{mol/L}$ (202 $\mu\text{g/dL}$).

Concentrations of glutamine are regularly elevated in patients with hyperammonemia, and concentrations of alanine are usually elevated as well, while concentrations of aspartic acid are elevated in some patients. These findings are nonspecific. They are not helpful in the differentiation of the different causes of hyperammonemia. They are potentially helpful in diagnosis, as sometimes an elevated level of glutamine is found in a patient who had not been expected to have hyperammonemia, and while concentrations of ammonia may vary from hour to hour, the elevated concentration of glutamine signifies a state in which there has been more chronic oversupply of ammonia. The transamination of pyruvic acid and that of oxalacetic acid and the subsequent amidation of glutamic acid all represent detoxification mechanisms in the attempt to handle excessive quantities of ammonia. An exception to the rule of elevation of glutamine in hyperammonemia appears to be propionic acidemia.

Amino acid analysis is not specifically useful in the diagnosis of OTC deficiency and carbamylphosphate synthetase deficiency, but in both of these conditions levels of citrulline and arginine may be low. They are distinguished by measurement of the excretion of orotic acid, which is increased in OTC deficiency, but not in carbamylphosphate synthetase deficiency or N-acetylglutamate synthetase deficiency [5, 6]. In OTC deficiency, this is a reflection of the accumulation of carbamylphosphate, which then leaves the mitochondria and follows the cytosolic pathway of pyrimidine synthesis (Figure 23.1). There may be so much orotic acid in the urine that it forms a white crystalline precipitate. In mice with OTC deficiency, calculi are found in the bladder [5–8].

Overproduction of pyrimidines leads to the presence of large amounts of uracil and increased amounts of uridine in the urine. In most patients with OTC deficiency, orotic aciduria is always present. However, we have studied one patient [6] with a partial variant in whom orotic acid excretion was not present when he was clinically well and could not be induced by means of a protein load, but was readily evident at the time of illness induced by infection.

TREATMENT OF HYPERAMMONEMIA

The treatment of the patient with hyperammonemia has many common features that are relevant to states of elevated ammonia, regardless of cause. In the acute hyperammonemic onset usually seen in infancy, all intake of protein or other sources of nitrogen is stopped. Water and electrolyte are provided intravenously, and anabolism is promoted by the administration of glucose. Pharmacologic approaches to therapy include the provision of arginine to keep the urea cycle supplied with sufficient ornithine to keep it running and the provision of alternate pathways for the excretion of waste nitrogen, such as sodium benzoate and sodium phenylacetate. Extracorporeal methods, such as hemodialysis, are often required in the acutely

hyperammonemic newborn. The most effective treatment of the acute hyperammonemic crisis that occurs in the classic neonatal disease is hemodialysis [9–11]. Exchange transfusion is not an effective modality in such an infant, but it may reduce levels enough to buy some time while the hemodialysis is being prepared, and it has been effective in some patients with transient hyperammonemia of the newborn [3]. Peritoneal dialysis is often recommended for hyperammonemic patients, and we would agree that it is effective in an older infant, child, or adult with hyperammonemia, but in our experience it has been unsatisfactory in the neonatal period [12]. Hemodialysis has been shown to be more effective than exchange transfusion, peritoneal dialysis or arteriovenous hemofiltration, but the logistics are such in most hospitals that this modality can seldom be mobilized promptly to meet the needs of a newly diagnosed newborn. This is an argument for transport of such an infant to an institution with experience in the rescue of such infants. Even so, the mechanics of hemodialysis in a tiny infant are formidable, and failure of the procedure is more common than the literature would imply. An advance in management has been the application of extracorporeal membrane oxygenation (ECMO) in the treatment of the hyperammonemic neonate [13].

The pharmacologic approach to the provision of alternate methods of waste nitrogen excretion represents a major advance in the management of hyperammonemia (Table 23.1 and Figure 23.3) [13–17]. The principle is that benzoate is effectively conjugated with glycine to form hippurate, which is efficiently excreted in the urine, and similarly phenylacetate is conjugated to form phenylacetylglutamine, and this compound is excreted in the urine; both provide pathways for getting rid of nitrogen that cannot be excreted as urea and would otherwise accumulate as ammonia. These measures have been employed along with exchange transfusion or peritoneal dialysis [3], but we have found pharmacologic therapy

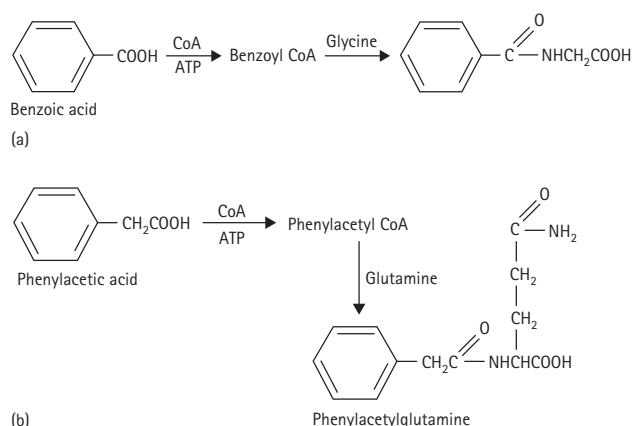


Figure 23.3 Structures of benzoic and phenylacetic acids and the mechanism of their excretion of waste nitrogen.

effective in patients in whom exchange transfusion and peritoneal dialysis were having little or no effect [6, 13].

Intravenous benzoate and phenylacetate are available commercially as a mixture from Ucyclid Pharma (Phoenix, AZ, USA). In Europe, most patients are treated with intravenous benzoate. Along with benzoate and/or phenylacetate, arginine is infused. This provides an essential amino acid in a patient with a complete block in arginine synthesis and provides ornithine substrate for any patient in whom there is not a complete block at carbamyl phosphate synthetase or ornithine transcarbamylase. It may be particularly useful as an effector of acetylglutamate synthetase and hence activator of carbamyl phosphate synthetase. It is particularly useful in patients with citrullinemia (Chapter 26) or argininosuccinic aciduria (Chapter 27). It is useful to start arginine alone if benzoate and phenylacetate are not immediately available. Supplies of benzoate, phenylacetate, and arginine should ideally be kept on hand and available to the neonatal intensive care

Table 23.1 Treatment of acute hyperammonemia in the neonatal period^a

Item	Action
1.	Nothing by mouth. No nitrogen intake. Start infusion of 10% glucose.
2.	Priming infusion containing 0.2–0.8 g/kg ^b of arginine hydrochloride (2–8 mL/kg of 10% arginine HCl), and 0.25 g/kg of sodium benzoate and 0.25 mg/kg sodium phenylacetate in approximately 30 mL/kg of 10% glucose over 1–2 hours. ^c
3.	Continuing infusion of 0.25–0.6 g/kg per 24 hours of sodium benzoate and sodium phenylacetate ^d and 0.2–0.8 g/kg of arginine ^e hydrochloride in 10% glucose and maintenance electrolyte in sufficient volume to be given over 24 hours.
4.	Alert teams for hemodialysis or hemofiltration, which must be instituted if pharmacological therapy is not successful, and is often required in the initial neonatal episode when the level of ammonia may be above three or four times the upper limit of normal by the time of diagnosis. Alert neurosurgeon as intracranial pressure monitor may be needed if intracranial pressure is increased. Mannitol is used for increased intracranial pressure.
5.	Pyridoxine 5 mg/kg and folic acid 0.1 mg/kg parenterally daily.

^aTreatment of the initial episode before definitive diagnosis is established.

^bHigher doses of arginine are employed in patients with citrullinemia (Chapter 26) and argininosuccinic aciduria (Chapter 27).

^cZofran may be given as an antiemetic (0.15 mg/kg intravenously) during the first 15 minutes of the priming infusion.

^dBoth sodium benzoate and phenylacetate may be omitted in some patients with citrullinemia and most patients with argininosuccinic aciduria, especially in the treatment of an intercurrent episode that is diagnosed promptly before the serum concentration of ammonia exceeds 200 μmol/L.

^eHigher doses of arginine may be employed in patients with citrullinemia (Chapter 26) and argininosuccinic aciduria (Chapter 27).

unit in anticipation of the diagnosis of such an infant. Often a patient is first recognized in a community hospital where benzoate and phenylacetate are not available, but arginine is. The arginine infusion should be started and the patient then transported to the tertiary care center. We have found that many patients will respond to arginine alone. This is often true in citrullinemia or argininosuccinic aciduria, but it is also true of patients with OTC deficiency in which a variant enzyme permits activity of the urea cycle as long as the cycle does not run out of ornithine substrate. Females with OTC deficiency, with one normal X chromosome seldom completely inactivated, can respond to arginine alone. The effect of arginine as an activator of carbamylphosphate synthetase should also be salubrious for patients with OTC deficiency, citrullinemia, and argininosuccinic aciduria. Operationally, for arginine as well as benzoate and/or phenylacetate, a priming infusion is followed by a regimen of continuous infusion until the ammonia has reached the normal range (Table 23.1). The use of mannitol for cerebral edema may also provide nitrogen excretion through diuresis in patients treated with benzoate, phenylacetate, and arginine.

The pharmacologic regimen is started on diagnosis in the acute neonatal hyperammonemic crisis and can be pursued while the dialysis team is being assembled. It may be effective in obviating the need for dialysis despite calculations that have been publicized [18]. It is usually effective in the management of recurrent episodes of hyperammonemia that occur in patients under therapy [16] at times of infection or other cause of catabolism, or vomiting, leading to an inability to continue oral treatment, because therapy of these episodes is generally initiated more promptly. It is also true that many patients successfully treated initially have died in later episodes of intercurrent hyperammonemia. The ideal approach to management is very early diagnosis so that hyperammonemia is prevented or treated before there is major elevation of the serum concentration of ammonia.

The drugs are supplied as concentrated solutions which would cause hyperosmolarity if infused directly. They are diluted in 30 mL/kg of 10 percent glucose for the priming infusion and later diluted in the 24-hour maintenance fluids, which are also 10 percent with respect to glucose, providing extra calories to spare catabolism. When the sensorium clears, and there is no vomiting, further calories can be provided by nasogastric tube in the form of polycose or Ross product Prophree or Mead Johnson product PDF usually diluted to supply 0.7 kcal/mL. Nitrogen intake is not resumed until the hyperammonemia has receded.

Long-term management of the hyperammonemic infant or child usually requires a combination of pharmacologic therapy and restriction of the intake of protein. Arginine is employed in doses of 0.4–0.7 g/kg. In OTC and carbamyl phosphate synthetase deficiencies, sodium benzoate is given in doses of 0.25–0.5 g/kg/day, and citrulline, which is more palatable, is employed as a source of ornithine in a dose of 0.17–0.25 g/kg. Oral sodium phenylbutyrate has

been employed as a source of phenylacetate in doses of 0.45–0.60 g/kg. It may be used as a substitute for benzoate, but many of our patients have found it unpalatable. A gastrostomy may be required. Protein is generally restricted to 0.7 g/kg and supplemented with 0.7 g/kg of a mixture of essential amino acids. We have felt that the optimal intake of whole protein should be determined in each patient and have found supplementation with relatively small amounts of alanine to be effective [19], but in urea cycle defects, extra essential amino acids are often necessary. Treatment is continued with oral folate 0.1 mg/kg and pyridoxine 5 mg. The former is employed to enhance transamination and the latter to promote the synthesis of glycine; both have elements in the success of therapy.

Chronic management of patients with urea cycle defects has also been effective using mixtures of the keto and hydroxy-acid analogs of essential amino acids [20]. These mixtures are no longer available in the United States, but there may still be a place for this anabolic approach to the removal of nitrogen, and long-term therapy in which keto acids were combined with benzoate in the successful treatment of a 30-month-old infant who at report was developing normally [15]. In this patient, nocturnal gavage was useful in the administration of the keto acids, arginine and benzoate.

N-acetylglutamate is an essential cofactor of the carbamyl phosphate synthase enzyme, and a small number of patients has been found with N-acetylglutamate synthase deficiency [21]. Carbamylglutamate is a structural analog of N-acetylglutamate, and Santiago Grisolia first suggested its use in hyperammonemia, caused by N-acetylglutamate synthetase deficiency (Nyhan, personal communication). This has turned out to be the case; treatment reduced levels of ammonia in the blood [22] and increased the incorporation of ^{15}N -ammonium chloride into urea [23]. Carbamylglutamate has also been used successfully to treat the acute hyperammonemia of methylmalonic acidemia [24]. Inhibition of N-acetylglutamate synthase is the mechanism by which propionyl CoA and similar compounds cause hyperammonemia [25].

Carbamylglutamate is available in Europe in 200-mg tablets for oral use, which limits its availability in acute hyperammonemic coma. It has been used via nasogastric tube. In the acute situation, a dose of 200 mg is appropriate. In the chronic management of N-acetylglutamate synthetase deficiency, doses of 15–100 mg/kg per day have been effective.

Follow up of 20 children treated with pharmacologic therapy who had a variety of defects of urea synthesis revealed a substantial 92 percent one-year survival, but 79 percent had significant developmental disability, and the mean IQ was 43 [26]. A significant and linear negative correlation was found between the duration of neonatal hyperammonemic coma and the IQ at 12 months. The peak level of neonatal hyperammonemia did not correlate significantly with later IQ. In this series of patients, there were seven infants with ornithine transcarbamylase

deficiency; two of the males died in hyperammonemic coma before one year of age. Computed tomography (CT) scans on the children in this series revealed considerable evidence of cerebral atrophy. There was a significant correlation between the presence of abnormalities seen on CT and the duration of neonatal hyperammonemic coma. The presence of abnormalities in the CT scan also correlated significantly with the ultimately determined IQ. Infants who were more than 5 days in hyperammonemic coma were invariably handicapped developmentally.

In a compilation of the Paris experience with the management and outcome of urea cycle defects [27], there were 121 patients with neonatal presentations, 661 of them OTC-deficient males, reflecting the severity of disease in this population in that all but one died promptly. Overall mortality of those with neonatal presentations was 84 percent. Girls with neonatal presentations of OTC deficiency also died in the neonatal period.

Of 96 late onset forms, OTC deficiency was also the most common. In this group, 18 percent died in the initial episode, but none once the diagnosis was established. It is clear from this experience that late onset forms are not uncommon. Neurologic manifestations were common in all the survivors.

REFERENCES

1. Grisolia S, Baguena R, Mayor F. *The Urea Cycle*. New York: John Wiley & Sons, 1976.
2. Lowenthal A, Mori A, Marescau B (eds). *Urea Cycle Diseases*. Advanced Experimental Medicine and Biology, vol. 153. New York: Plenum Press, 1982.
3. Ballard RA, Vinocur B, Reynolds JW et al. Transient hyperammonemia of the preterm infant. *New Engl J Med* 1978; **299**: 920.
4. Marsden D, Sege-Peterson K, Nyhan WL et al. An unusual presentation of medium-chain acyl coenzyme A dehydrogenase deficiency. *Am J Dis Child* 1992; **146**: 1459.
5. Oizumi J, Ng WG, Koch R et al. Partial ornithine transcarbamylase deficiency associated with recurrent hyperammonemia, lethargy and depressed sensorium. *Clin Genet* 1984; **25**: 538.
6. Christadolu J, Qureshi IA, McInnes RR, Clarke JTR. Ornithine transcarbamylase deficiency presenting with stroke-like episodes. *J Pediatr* 1993; **122**: 423.
7. Demars R, Levan SL, Trend BL et al. Abnormal ornithine carbamyl-transferase in mice having the sparse-fur mutation. *Proc Natl Acad Sci USA* 1972; **73**: 1693.
8. Snyderman SE, Sansaricq C, Phansalkar SV et al. The therapy of hyperammonemia due to ornithine transcarbamylase deficiency in a male neonate. *Pediatrics* 1975; **56**: 65.
9. Donn SM, Swartz RD, Thoene JG. Comparison of exchange transfusion, peritoneal dialysis and hemodialysis for the treatment of hyperammonemia in an anuric newborn infant. *J Pediatr* 1979; **95**: 67.
10. Wiegand C, Thompson T, Bock GH et al. The management of life-threatening hyperammonemia: a comparison of several therapeutic modalities. *J Pediatr* 1980; **96**: 142.
11. Kiley JE, Pender JC, Welsch HF, Welsch CS. Ammonia intoxication treated by hemodialysis. *N Engl J Med* 1958; **259**: 1156.
12. Nyhan WL, Wolff J, Kulovich S, Schumacher A. Intestinal obstruction due to peritoneal adhesions as a complication of peritoneal dialysis for neonatal hyperammonemia. *Eur J Pediatr* 1985; **143**: 211.
13. Summar M. Current strategies for the management of neonatal urea cycle disorders. *Pediatrics* 2001; **138**: S31.
14. Batshaw ML, Brusilow SW. Treatment of hyperammonemic coma caused by inborn errors of urea synthesis. *J Pediatr* 1980; **97**: 893.
15. Guibaud P, Baxter P, Bourgeois J et al. Severe ornithine transcarbamylase deficiency. Two and a half years' survival with normal development. *Arch Dis Child* 1984; **59**: 477.
16. Brusilow SW, Danney M, Waber LJ et al. Treatment of episodic hyperammonemia in children with inborn errors of urea synthesis. *N Engl J Med* 1983; **310**: 1620.
17. Batshaw ML, Brusilow S, Waber L et al. Treatment of inborn errors of urea synthesis: activation of alternative pathways of waste nitrogen synthesis and excretion. *N Engl J Med* 1982; **306**: 1387.
18. Brusilow SW, Horwich AL. Urea cycle enzymes. In: Scriver CR, Beaudet AL, Sly WS et al. (eds). *The Metabolic and Molecular Bases of Inherited Disease*. New York: McGraw Hill, 1976: 1187-232.
19. Wolff J, Kelts DG, Algert S et al. Alanine decreases the protein requirements of infants with inborn errors of amino acid metabolism. *J Neurogenet* 1985; **2**: 41.
20. Thoene J, Batshaw M, Spector E et al. Neonatal citrullinemia: treatment with keto-analogues of essential amino acids. *Pediatrics* 1977; **90**: 218.
21. Bachmann C, Colombo JP, Jaggi K. N-acetylglutamate synthetase (NAGS) deficiency: diagnosis, clinical observations and treatment. *Adv Exp Med Biol* 1977; **153**: 39-45.
22. Caldovic L, Tuchman M. N-acetylglutamate and its changing role through evolution. *Biochem J* 2003; **372**: 279-90.
23. Caldovic L, Morizono H, Daikhin Y. Restoration of urea genesis in N-acetylglutamate synthase deficiency by N-carbamylglutamate. *J Pediatr* 2004; **145**: 552-4.
24. Gebhardt B, Vlaho S, Fischer D et al. N-carbamylglutamate enhances ammonia detoxification in a patient with decompensated methylmalonic aciduria. *Mol Genet Metab* 2003; **79**: 303-4.
25. Coude FX, Sweetman L, Nyhan WL. Inhibition by propionyl-coenzyme A of N-acetylglutamate synthetase in rat liver mitochondria. A possible explanation for hyperammonemia in propionic and methylmalonic acidemia. *J Clin Invest* 1979; **64**: 1544-51.
26. Msall M, Batshaw ML, Suss R et al. Neurologic outcome in children with inborn errors of urea synthesis. *N Engl J Med* 1984; **310**: 1500.
27. Nassogne M, Heron B, Touatt G et al. Urea cycle defects: management and outcome. *J Inher Metab Dis* 2005; **28**: 407.

Ornithine transcarbamylase deficiency

Introduction	197	Treatment	201
Clinical abnormalities	197	References	202
Genetics and pathogenesis	199		

MAJOR PHENOTYPIC EXPRESSION

Potentially lethal hyperammonemic coma in the male and varying expression in the female, consistent with X-linked transmission; convulsions; elevated concentrations of glutamine and alanine; orotic aciduria; and defective activity of hepatic ornithine transcarbamylase.

INTRODUCTION

The most classic of the infantile urea cycle presentations is that of ornithine transcarbamylase (OTC) deficiency in the male. Onset is in the neonatal period with coma and or convulsions, and in the absence of effective intervention, it is rapidly fatal. A small number of males has variant enzymes and a milder and later presentation, but hyperammonemia can still be fatal, even in adulthood. Females who have two X chromosomes have varying phenotypes depending on the proportion of active and inactive X chromosomes.

The enzyme ornithine transcarbamylase or ornithine carbamoyltransferase (OCT) (EC 2.1.33) is found almost exclusively in liver. There is less activity in small intestine and a small amount in brain. It catalyzes the conversion of ornithine and carbamylphosphate to citrulline (see Figure 23.1 in [Chapter 23](#)). The hepatic enzyme is located in the mitochondria [1]. The enzyme is a trimer in which identical subunits have a molecular weight of about 38 kDa [2–4]. The OTC enzyme is synthesized in the cytosol and transported to its mitochondrial site of activity. The protein synthesized contains an N-terminal signal peptide that is specifically recognized by a receptor complex in the outer mitochondrial membrane [5]. After translocation across the membranes, proteolytic processing by two peptidases yields the mature protein [6]. Once imported into the mitochondria, the OTC subunits require folding to form the active trimer, and this process is mediated by molecular chaperones.

Defective activity of the enzyme is readily demonstrable

in biopsied liver [7–10]. The gene on the X-chromosome codes for the precursor protein that is imported after translation into mitochondria. The human OTC precursor cDNA has been isolated and cloned [11]. It is localized to band p2.1 in the short arm [12], just proximal to the locus for Duchenne muscular dystrophy. The genes for glycerol kinase, adrenal insufficiency, chronic granulomatous disease, Norrie disease, and retinitis pigmentosa are all in this area, and a number of contiguous gene syndromes have resulted from deletions. Large deletions account for about 16 percent of mutations in affected males [13]. Another 10 percent have point mutations in a *TaqI* recognition site in exon 5 in (TCGA) which changes the code for arginine at position 109 of the mature protein and changes it to either glutamine or a stop codon and reduces enzyme activity to 1 percent of normal or less [14, 15]. Many other point mutations have established an enormous heterogeneity. By 1996, some 90 mutations had been documented [15].

CLINICAL ABNORMALITIES

Ornithine transcarbamylase deficiency in its usual presentation in the neonatal male infant ([Figure 24.1](#)) provides the classic picture of a defect in the urea cycle (see Figure 23.1) [16, 17]. Prior to the recent development of pharmacologic approaches to the removal of waste nitrogen using benzoate and phenylacetate, this disorder was always fatal and usually within just a few days of birth. The mortality is still high.



Figure 24.1 JN: A male infant with ornithine transcarbamylase deficiency after recovery from neonatal hyperammonemia. The site of a Tenckhoff catheter that had been unsuccessfully used for peritoneal dialysis is evident on the abdomen.

Affected infants are often thought to have sepsis. Occasionally, the diagnosis of hyperammonemia is made once blood culture is found to be negative. On the other hand, we have encountered neonates with urea cycle defects who have actually had sepsis, further confusing the diagnosis. It is therefore advisable to obtain blood for ammonia in any infant in coma. A bulging fontanel may suggest intracranial hemorrhage, but it is more often the result of cerebral edema, and computed tomography (CT) scan should resolve the issue.

The infant appears normal at birth and remains so during a period of hiatus, which may be as short as a few hours and is seldom longer than 48 hours. He then begins to be lethargic and to refuse feedings. Grunting or rapid respirations may occur, and there may be a respiratory alkalosis. Convulsions may be generalized, and the electroencephalogram (EEG) is usually abnormal. The infant may have hypertonia, but there is progression to a deep coma that is indistinguishable from surgical anesthesia. Ultimately, the infant stops breathing, and unless he is intubated and artificially ventilated, he dies. Despite initial improvement following dialysis or other interventions, which decrease ammonia concentrations, most of these patients have died. Those surviving because of successful pharmacologic therapy have usually had severely impaired mental development, if the initial hyperammonemic coma has been profound and prolonged. Most have had recurrent hyperammonemic crises at times of catabolism induced by intercurrent illness, and each further episode appears to worsen the prognosis for mental development. If, in a family at risk, the diagnosis can be made prior to hyperammonemic coma and the patient prevented from ever having such an episode, then the development of the nervous system should be normal, but patients fitting these criteria among males with the classic form of OTC deficiency, have not yet been described. It still may

be the rule, rather than the exception, for those patients who survive the neonatal attack to die in infancy in a subsequent hyperammonemic episode that accompanies an acute infection.

The major metabolic characteristic of patients with ornithine transcarbamylase deficiency is hyperammonemia. Levels found in the classic neonatal form of the disease are usually over $700 \mu\text{mol/L}$ ($1000 \mu\text{g/dL}$). Coma is generally present when the concentration exceeds $250 \mu\text{mol/L}$ ($400 \mu\text{g/dL}$). In infants dying of the disease, levels may range from 400 to $1700 \mu\text{mol/L}$ (600 – $2500 \mu\text{g/dL}$). In the presence of levels over $300 \mu\text{mol/L}$ ($500 \mu\text{g/dL}$), one sees fixed dilated pupils and complete apnea. Cerebral edema occurs in some patients at these levels. Normal neonatal ammonia is $50 \mu\text{mol/L}$. On the other hand, it is not uncommon to observe levels up to $100 \mu\text{mol/L}$ in normal infants. Problems in obtaining and handling blood samples invariably raise levels, and exercise such as squeezing a ball, can raise the level to $150 \mu\text{mol/L}$ [18]. Thus, a normal level eliminates hyperammonemia, but an abnormal level that does not fit with clinical findings may have to be repeated or confirmed by the presence of an elevated glutamine. Consensus has not been reached as to a specific neonatal level that should prompt intravenous therapy. Consensus was also not reached on the necessity for intravenous therapy for mild elevations of ammonia in chronically treated patients [18].

In the female, the range of variation in symptomatology



Figure 24.2 TC: A two-year-old girl with ornithine transcarbamylase deficiency. During infancy, she had many episodes of hyperammonemia despite therapy with arginine, benzoate, and phenylacetate, but each was treated promptly, and cognitive development was good. Nevertheless, she died in a subsequent hyperammonemic episode.

is very great (Figure 24.2) [19–23]. This is consistent with varying degrees of inactivation of the normal X chromosome called for by lyonization as a random process. Among these patients, delay in diagnosis is common. In a series of 13, the mean interval from onset of symptoms to diagnosis was 16 months, and the range was 1–142 months [19]. At one end of the spectrum is a small number of female infants with an overwhelming clinical picture indistinguishable from that of the male and leading to death in infancy. Others, only somewhat less severe, have had episodes of recurrent hyperammonemia followed by death in childhood. Others have had recurrent vomiting beginning in infancy or as late as nine years of age [20]. This condition should be included in the differential diagnosis of cyclic vomiting. Attacks may be accompanied by headache, slurring of speech, or screaming. An attack may also present with ataxia. A patient with recurrent episodes of intense headache and ataxia may appear to have migraine. During the attack, the patient may display muscular rigidity or opisthotonus. There may be convulsions. Any patient with ornithine transcarbamylase deficiency and symptomatic attacks of hyperammonemia may develop coma, and this may go on to death. Hepatomegaly is seen in some patients, and there may be abnormalities in liver function tests. In an older child seen for the first time with hyperammonemia, these findings may be thought at first to represent primary disease of the liver. OTC deficiency was on occasion initially diagnosed as Reye syndrome [21]. Attacks may be precipitated by a large intake of protein, infection, surgery, or immunization. Impaired mental development may be progressive with further episodes.

At the other end of the spectrum are women who are completely asymptomatic. They are found to be heterozygous for deficiency of ornithine transcarbamylase because a male son or other relative is found to have the classic disease. Some of these women have a dislike of protein foods and thus have not stressed the system. Others appear to have no trouble with protein. Nevertheless, a careful study of the IQ scores of heterozygotes, identified by the urinary excretion of orotic acid following a protein load, were found to be 6 to 10 points lower than in the controls, who were relatives in whom the orotic acid test was negative [23].

Some males have been reported in whom there was a much milder or late onset clinical phenotype, similar to that described in females. These patients appear to represent variants different from the classic one in that they have a defect in the enzyme that leads to partial activity [24–32]. Prominent symptoms are recurrent vomiting, lethargy, irritability, and protein avoidance. A patient with one of these variants may have normal development and may progress normally in school [26, 28]. The disease may present in adulthood [30]. It may present with bizarre behavior [30, 31]. In fact, recurrent episodes of bizarre behavior may be the only symptoms of this type of OTC deficiency [30]. Measurement of orotic acid and orotidine, even after allopurinol or a protein load, failed to elucidate

the diagnosis in this patient, but a high protein diet led to orotic aciduria. In the late onset male, as in the symptomatic female, the disease is nevertheless potentially lethal, and death may ultimately occur in a hyperammonemic episode even after a number of symptom-free years. In a series of 21 male patients who presented at ages ranging from two months to 44 years, 43 percent died [32]. The mean interval from the age at onset to that at diagnosis was 8.8 months, with a range of up to 54 months. An initial diagnosis of Reye syndrome was made in 52 percent of the patients. Death with cerebral edema was the result of an initial episode following relatively trivial surgery in a 52-year-old man [33]. The diagnosis was made by mutational analysis in his heterozygous daughter. Prenatal diagnosis of her affected twin sons led to effective management. Among complications of OTC deficiency, sudden strokes have been reported [34]. This has also been seen in carbamylphosphate synthetase (CPS) deficiency and an enlarging group of metabolic diseases.

The diagnosis is suspected on the basis of the blood level of ammonia and suspicions are confirmed by elevations in glutamine and often alanine. An algorithmic approach to the exclusion of nonurea cycle causes of hyperammonemia and the differentiation among specific urea cycle defects (Chapter 23) identifies those disorders in which a specific amino acid, such as citrulline, is elevated. Elevated excretion of orotic acid then differentiates OTC deficiency from that of CPS in which orotic acid levels are normal. Definitive diagnosis of OTC deficiency usually requires assay of the enzyme in biopsied liver, but mutation analysis when positive obviates this. If the mutation in a family is known, analysis of the DNA will make the diagnosis, but mutational analysis may also be successful in the absence of a family mutation. Disorders of fatty acid oxidation may have an identical Reye-like presentation and meet all of the conditions for a diagnosis of OTC deficiency, except that the enzyme activity of the liver is normal [35].

GENETICS AND PATHOGENESIS

The gene for ornithine transcarbamylase is located on the X chromosome [11, 12]. The disease is expressed as an X-linked dominant. Thus, in females in whom a major proportion of cells contain the inactivated normal X chromosome, the severity of disease may be as great as in the homozygous male. At the other end of the spectrum, even asymptomatic female heterozygotes may have lower IQs than their homozygous normal relatives [23].

The molecular defect in ornithine transcarbamylase is readily detected in the liver. This enzyme is also expressed in intestinal mucosa, and therefore the diagnosis has been made by assay of tissue obtained by rectal or duodenal biopsy [36]. However, the gold standard is the assay of biopsied liver.

In males with the lethal neonatal disease, enzyme activity is virtually absent [7–10]. In males with the partial variants,

levels range from 5 to 25 percent of normal [26, 27, 37]. Some of these patients have been reported to have virtually zero activity [38, 39], but this is not likely to reflect the level of activity that is functional *in vivo*. Abnormal proteins tend to be unstable and break down readily under conditions of *in vitro* assays. In symptomatic heterozygous females, levels of activity have ranged from 4 to 25 percent of normal [8]. As much as 97 percent of normal activity has been found in known heterozygous mothers of affected children. Because the female is a mosaic of hepatocytes, the level of activity found in a biopsy may not necessarily reflect the *in vivo* activity of their conglomerate, but the diagnosis should nevertheless be clear. In some males with partial variants, the kinetic properties of the enzyme have been studied [40–42]. These variant enzymes have been found to have alterations in the K_m for ornithine or carbamyl phosphate or the optimal pH. The use of antibody prepared against the normal human enzyme has shown no cross-reacting material (CRM) in most hemizygous males, but a few were CRM-positive [42].

The cloning of the gene for OTC [11] and the elucidation of its structure [43] have permitted the identification of more than 150 different mutations more [15, 44–46]. Some 42 percent of mutations were associated with acute neonatal diseases; 21 percent were in later onset males [44] and 37 percent in manifesting females. The incidence of new mutation in the genesis of girls with OTC deficiency has been much greater than in boys [46], suggesting that mutation is more common in sperm than in ova. Among males, only two of 28 had sporadic mutations, while some 95 percent inherited their mutations from their mothers. Among females, only 20 percent inherited their mutations from their mothers. A mother found not to carry the mutant allele in a child could have gonadal mosaicism. This was found in a somatically normal woman who produced multiple affected males [47]. Fortunately, this is very rare.

A very great amount of heterogeneity has been identified. Most families have had unique mutations. Large deletions of an exon or more were found in 7 percent of families and small deletions or insertions in 15 percent [13]. Most families have had point mutations; of families with nucleotide substitutions, less than half had mutations seen in at least one other family. All but two of these recurrent mutations occurred in CpG dinucleotides, and the mutations were spread over many of the CpG dinucleotides. The most frequent mutation was the R129H mutation in which a G to A change at the end of exon 4 replaces an arginine with a histidine. This relatively neutral substitution causes abnormal splicing and very low activity [48]. The identical mutation occurs in the *spf-ash* mouse [49]. Some correlation of phenotype and genotype are emerging. For instance, a Tyr 167 stop [50] led to truncation with loss of the ornithine binding residues, a complete loss of activity of OTC and hyperammonemic death at 4 days of life. A mutation reported [51] in the leader peptide region, which would lead to failure to enter the mitochondria, led to a severe neonatal phenotype and an absence of OTC

activity in liver. Mutations have been distributed across the entire gene. Mutations at consensus splice sites caused the neonatal phenotype [44]. Overall, in patients with proven deficiency of enzyme activity, only 80 percent of mutations have been found [15].

Defective activity of the hepatic enzyme has been documented in heterozygotes, and histochemical assay of the enzyme demonstrated the presence of two populations of hepatic cells, one normal and one deficient in the activity of ornithine transcarbamylase deficiency [52], consistent with the Lyon hypothesis. Heterozygosity has also been documented by assay of the enzyme in duodenal mucosa [53]. This method has failed to detect some known heterozygotes.

Heterozygosity has been detected by assay of the urine for orotic acid following a load of 1 g/kg of protein [23, 53, 54]. Urine is usually collected in three 4-hour aliquots following the load. More reproducible results may be obtained using an alanine load [55]. However, it is clear that the 0.7 g/kg dose that was initially employed was too large. Such a dose can lead to alarming symptoms in a heterozygote. It also may overwhelm the system in a normal and lead to a false-positive diagnosis of heterozygosity. We used 0.4 g/kg along with a very sensitive method for orotic acid using stable isotope dilution [55], and selected ion monitoring gas chromatography-mass spectrometry [56]. Both protein and alanine loading have largely been supplanted now by the allopurinol test [57]. Allopurinol inhibits the decarboxylation of orotidine monophosphate (orotidylate) (OMP) (see Figure 23.1) via reaction of its phosphorylated oxidation product on the decarboxylase. When OMP accumulates it is reflected in the urine in the excretion of orotidine and orotic acid. The specificity of the test was reported to be greater when orotidine was measured than when orotic acid was measured [57]. If the mutation is known, it can be effectively employed in heterozygote detection, but the enormous amount of variation in OTC deficiency often tends to make this impractical.

The ability to determine carrier status by mutational analysis (Figure 24.3) has made it clear that the allopurinol test, which was designed in families of patients with classic neonatal OTC deficiencies, is not reliable in the families of male patients with variant phenotypes [58, 59]. In a series of 18 asymptomatic carriers, three genotypic heterozygotes were not identified by allopurinol testing [60]. In some instances, the nature of the mutation cannot be identified. In these families, linkage analysis is sometimes useful, taking advantage of restriction fragment length polymorphism. The possibility of gonadal mosaicism should be remembered in any diagnosis that a woman is not a carrier. Heterozygote detection has also been carried out for diagnostic purposes in a symptomatic girl by the ratio of transfer of administered ^{15}N -glutamine to ^{15}N -urea (^{15}N -U/G ratio) [61].

Prenatal diagnosis has been carried out by assay of the enzyme in biopsied fetal liver [62]. The risk of fetal loss makes assay for the gene much more satisfactory. Prenatal

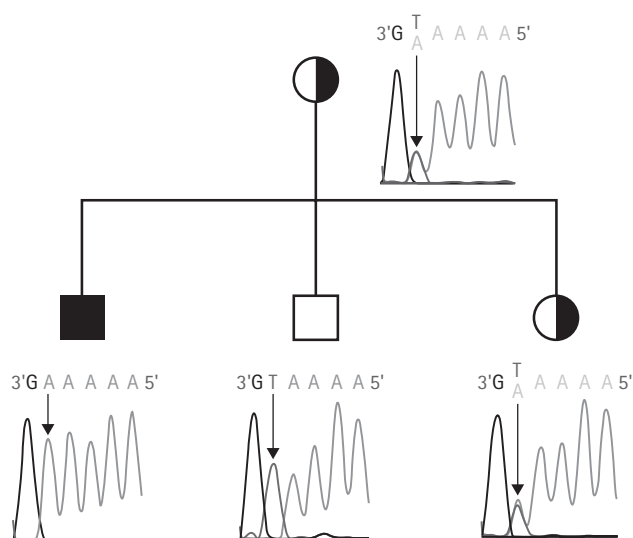


Figure 24.3 Kindred in which an A to T mutation in exon 9 of the ornithine transcarbamylase gene specified a leucine 301 phenylalanine replacement in the enzyme was identified in three individuals in two generations. The proband had a variant late onset phenotype [60]. In each of the heterozygotes, testing for orotic acid and/or orotidine after allopurinol failed to detect heterozygosity.

diagnosis has been carried out by assay for the *TaqI* cleavage site [63], and by assay for mutations known in probands. Certainly, if precise mutation analysis is available, it should always be employed in prenatal diagnosis.

TREATMENT

The treatment of the acute hyperammonemic episode, long-term management, and the results of treatment have been set out in detail in [Chapter 23](#). In the neonatal hyperammonemia of OTC deficiency, the most vigorous intervention is required and even then it is often impossible to avoid death or severe neurologic disability. In neonatal onset urea cycle defects, best results have been achieved in patients, especially female with OTC deficiency, in whom prenatal diagnosis is made, but even in these patients the risk of sudden death or disability is always present.

Most hyperammonemic infants with OTC deficiency require hemodialysis [64], and extracorporeal membrane oxygenation (ECMO) is probably preferable. Pharmacologic

therapy with intravenous sodium benzoate/phenylacetate and arginine are initiated promptly and pursued vigorously. The management of intercurrent episodes of hyperammonemia in a patient rescued from the initial neonatal episode is similar, but it is hoped that treatment may be initiated promptly enough to abort the episode without the need for dialysis.

In OTC deficiency, the priming infusion contains sodium benzoate and phenylacetate 0.25 g/kg each and 0.2 g/kg of arginine.HCl (2 mL/kg of a 10 percent solution). The sustaining infusion given over the next 24 hours has the same content of each.

In chronic management of OTC deficiency ([Table 24.1](#)), citrulline is substituted for arginine, as it is more palatable. Most patients are also treated with phenylbutyrate [65] as a source of phenylacetate. To many, phenylbutyrate is so unpalatable that a gastrostomy tube is required in order to avoid poor compliance, and even then patients complain of a taste. For these reasons, sodium benzoate is preferred by some patients and some authorities. Most patients require restriction of the intake of protein, and most receive mixtures of essential amino acids to minimize the intake of nonessential nitrogen. Protein restriction and the use of benzoate/phenylbutyrate and deficiency of essential amino acids may lead to Kwashiorkor. Acrodermatitis-like skin lesions have been reported [66]. Complexities of management of OTC deficiency were discussed in a patient diagnosed prenatally with a deletion who developed neurologic disease and anasarca, despite exemplary management and avoidance of hyperammonemia. Whole genome sequencing led to the diagnosis of a contiguous gene deletion involving chronic granulomatous disease, retinitis pigmentosa, and McLeod syndrome [67]. The authors stressed the need for detailed genetic analysis.

The prognosis in OTC deficiency is always guarded. In the neonatal onset hyperammonemic phenotype, virtually all have died (Saudubray *et al.*, personal communication, 1996). Later onset patients are nearly all female. Many of these have also died or developed major neurologic disability. These considerations have led to the use of orthotopic transplantation of the liver (Saudubray *et al.*, personal communication, 1996) [61, 68, 69]. Experience has been that following transplantation, levels of ammonia are no longer a problem, and that protein restriction and medication to handle waste nitrogen are no longer required. Balancing the risks of the transplantation becomes a factor in later onset girls, but there is little question in patients with infantile presentations, that the risk of the disease

Table 24.1 Chronic management of ornithine transcarbamylase deficiency

Citrulline	NaPhenylbutyrate ^a	(g/kg per 24 hours) ^a		
		NaBenzoate	Whole protein	Essential amino acids
0.17–0.25	0.45–0.60	0.25–0.50	0.7	0.7

^aNaBenzoate or NaPhenylbutyrate are given, usually not both, although some patients have been treated with both.

is much higher. Technical problems make it difficult to transplant liver much before the age of one year. In this way, most neonatal onset males would be excluded. The use of living donor transplantation and split liver or auxiliary liver transplantation should decrease the time waiting for transplantation and increase the possibility of application before the intervention of death or severe disability. Successful liver transplantation was reported in a 60-day-old patient with this disease [68]. At 13 months of age, his Bailey Scale of Infant Development index was less than 50 percent, and magnetic resonance imaging (MRI) revealed cortical atrophy. In three boys transplanted between 40 and 223 days of age [70], urea production was normal as were levels of ammonia. Two of the three performed at age-approved levels. Liver cell transplantation has been shown now in a small number of patients to improve metabolism of waste nitrogen sufficiently to buy time for an ultimate liver transplantation. At removal of the recipient liver, viable islands of transplanted cells have been found.

REFERENCES

1. Merker HJ. Electron microscopic demonstration of ornithine carbamyl transferase in rat liver. *Histochemia* 1969; **17**: 83.
2. Marshall M, Cohen PP. Ornithine transcarbamylase from *Streptococcus faecalis* and bovine liver: I. Isolation and subunit structure. *J Biol Chem* 1972; **247**: 1961.
3. Clarke S. The polypeptides of rat liver mitochondria: identification of a 36 000 dalton polypeptide as the subunit of ornithine transcarbamylase. *Biochem Biophys Res Commun* 1976; **71**: 1118.
4. Marshall M. Ornithine transcarbamylase from bovine liver. In: Grisolia S, Baguena R, Mayor F (eds). *The Urea Cycle*. New York: John Wiley & Sons, 1976: 169.
5. Horwich AL, Kalousek F, Fenton WA *et al*. Targeting of pre-ornithine transcarbamylase to mitochondria: definition of critical regions and residues in the leader peptide. *Cell* 1986; **44**: 451.
6. Kalousek F, Hendrick JP, Rosenberg LE. Two mitochondrial matrix proteases act sequentially in the processing of mammalian matrix enzymes. *Proc Natl Acad Sci USA* 1988; **85**: 7536.
7. Campbell AGM, Rosenberg LE, Snodgrass PJ, Nuzum CT. Ornithine transcarbamylase deficiency. A cause of lethal neonatal hyperammonemia in males. *N Engl J Med* 1973; **288**: 1.
8. Short EM, Conn HQ, Snodgrass PJ *et al*. Evidence for X-linked dominant inheritance of ornithine transcarbamylase deficiency. *N Engl J Med* 1973; **288**: 7.
9. Kang ES, Snodgrass PJ, Gerald PS. Ornithine transcarbamylase deficiency in the newborn. *J Pediatr* 1973; **82**: 642.
10. Goldstein AS, Hoogenraad HJ, Johnson JD *et al*. Metabolic and genetic studies of a family with ornithine transcarbamylase (OTC) deficiency. *Pediatr Res* 1974; **8**: 5.
11. Horwich AL, Fenton WA, Williams KR *et al*. Structure and expression of a complementary DNA for the nuclear coded precursor of human mitochondrial ornithine transcarbamylase. *Science* 1984; **224**: 1068.
12. Lindgren V, De Martinville B, Horwich AL *et al*. Human ornithine transcarbamylase locus mapped to band Xp211 near the Duchenne muscular dystrophy locus. *Science* 1984; **226**: 698.
13. Tuchman M, Morizono H, Rajagopal BS *et al*. The biochemical and molecular spectrum of ornithine transcarbamylase deficiency. *J Inher Metab Dis* 1998; **21**(Suppl.): 40.
14. Maddalena A, Edward SJ, O'Brien WE, Nussbaum RL. Characterization of point mutations in the same arginine codon in three unrelated patients with ornithine transcarbamylase deficiency. *J Clin Invest* 1988; **82**: 1353.
15. Tuchman M, Plante RJ, Garcia-Perez MG *et al*. Relative frequency of mutations causing ornithine transcarbamylase deficiency in 78 families. *Hum Genet* 1996; **96**: 274.
16. Levin B, Oberholzer VG, Sinclair L. Biochemical investigations of hyperammonaemia. *Lancet* 1969; **2**: 170.
17. Brusilow SW, Batshaw ML, Waber L. Neonatal hyperammonemic coma. *Adv Pediatr* 1982; **29**: 69.
18. The urea cycle disorders. Conference proceedings of a consensus conference for the management of patients with urea cycle disorders. *J Pediatr* 2001; **138**: S1–S80.
19. Rowe PC, Newman SL, Brusilow SW. Natural history of symptomatic partial ornithine transcarbamylase deficiency. *N Engl J Med* 1986; **314**: 541.
20. Russell A, Levin B, Oberholzer VG, Sinclair L. Hyperammonaemia. A new instance of an inborn enzymatic defect of the biosynthesis of urea. *Lancet* 1962; **2**: 699.
21. Rowe PC, Valle D, Brusilow SW. Inborn errors of metabolism in children referred with Reye's syndrome. *J Am Med Assoc* 1988; **260**: 3167.
22. Arn PH, Hauser ER, Thomas GH *et al*. Hyperammonemia in women with a mutation at the ornithine carbamoyl transferase locus: a cause of post-partum coma. *N Engl J Med* 1990; **322**: 1652.
23. Batshaw ML, Roan Y, Jung AL *et al*. Cerebral dysfunction in asymptomatic carriers of ornithine transcarbamylase deficiency. *N Engl J Med* 1980; **302**: 482.
24. Cathelineau L, Briand P, Petit F *et al*. Kinetic analysis of a new ornithine transcarbamylase variant. *Biochim Biophys Acta* 1980; **614**: 40.
25. Haan EA, Banks DM, Hoogenraad NJ, Roger JG. Hereditary hyperammonemic syndromes – a six year experience. *Aust Pediatr J* 1979; **15**: 142.
26. Oizumi J, Ng WG, Koch R *et al*. Partial ornithine transcarbamylase deficiency associated with recurrent hyperammonemia lethargy and depressed sensorium. *Clin Genet* 1984; **25**: 538.
27. MacLeod P, Mackenzie S, Scriver CR. Partial ornithine carbamyl transferase deficiency: an inborn error of the urea cycle presenting as orotic aciduria in a male infant. *Can Med Assoc J* 1972; **107**: 405.
28. Yudkoff M, Yang W, Snodgrass PJ, Segal S. Ornithine transcarbamylase deficiency in a boy with normal development. *J Pediatr* 1980; **96**: 441.
29. Tallan HH, Shaffner F, Taffet SL *et al*. Ornithine carbamoyl

- transferase deficiency in an adult male: significance of hepatic ultrastructure in clinical diagnosis. *Pediatrics* 1983; **71**: 224.
30. Dimagno EP, Lowe JE, Snodgrass PJ, Jones JD. Ornithine transcarbamylase deficiency – a cause of bizarre behavior in man. *N Engl J Med* 1986; **315**: 744.
 31. Spada M, Guardamagna O, Rabier D *et al*. Recurrent episodes of bizarre behavior in a boy with ornithine transcarbamylase deficiency: diagnostic failure of protein loading and allopurinol challenge tests. *J Pediatr* 1994; **125**: 249.
 32. Finkelstein JE, Hauser ER, Leonard CO, Brusilow SW. Late-onset ornithine transcarbamylase deficiency in male patients. *J Pediatr* 1990; **117**: 897.
 33. Lien J, Nyhan WL, Barshop BA. Fatal initial adult-onset presentation of urea cycle defect. *Arch Neurol* 2007; **64**: 1777.
 34. Christadolu J, Qureshi IA, McInnes RR, Clarke JTR. Ornithine transcarbamylase deficiency presenting with stroke-like episodes. *J Pediatr* 1993; **122**: 423.
 35. Marsden D, Sege-Peterson J, Nyhan WL *et al*. An unusual presentation of medium-chain acyl coenzyme A dehydrogenase deficiency. *Am J Dis Child* 1992; **146**: 1459.
 36. Matsushima A, Orii T. The activity of carbamylphosphate synthetase I (CPS I) and ornithine transcarbamylase (OTC) in the intestine and the screening of OTC deficiency in the rectal mucosa. *J Inher Metab Dis* 1981; **4**: 83.
 37. Cathelineau L, Saudubray JM, Navarro J, Polonovski C. Transmission par le chromosome X du gene de structure 1-ornithine carbamyl transferase. Etude de trois familles. *Ann Genet* 1973; **16**: 173.
 38. Krieger I, Snodgrass PJ, Roskamo J. Atypical clinical course of ornithine transcarbamylase deficiency due to a new mutant (comparison with Reye's disease). *J Clin Endocrinol Metab* 1979; **48**: 338.
 39. Matsuda I, Arashima S, Nambu H *et al*. Hyperammonemia due to a mutant enzyme of ornithine transcarbamylase. *Pediatrics* 1971; **48**: 595.
 40. Cathelineau L, Saudubray JM, Polonovski C. Ornithine carbamyl transferase: the effects of pH on the kinetics of a mutant human enzyme. *Clin Chim Acta* 1972; **41**: 305.
 41. Cathelineau L, Saudubray JM, Polonovski C. Heterogeneous mutations of the structural gene of human ornithine carbamyl transferase as observed in five personal cases. *Enzyme* 1974; **18**: 103.
 42. Briand P, Francois B, Rabier D, Cathelineau L. Ornithine transcarbamylase deficiencies in human males. Kinetic and immunochemical classification. *Biochim Biophys Acta* 1981; **704**: 100.
 43. Hata A, Tsuzuki T, Shimada K *et al*. Structure of the human ornithine transcarbamylase gene. *J Biochem* 1988; **103**: 302.
 44. Tuchman M. Mutations and polymorphisms in the human ornithine transcarbamylase gene. *Hum Mut* 1993; **2**: 174.
 45. Tuchman M, Plante RJ. Mutations and polymorphisms in the human ornithine transcarbamylase gene: update addendum. *Hum Mutat* 1995; **5**: 293 (review).
 46. Tuchman M, Matsuda I, Munnich A *et al*. Proportions of spontaneous mutations in males and females with ornithine transcarbamylase deficiency. *Am J Med Genet* 1995; **55**: 67.
 47. Bowling F, McGown I, McGill S *et al*. Maternal gonadal mosaicism causing ornithine transcarbamylase deficiency. *Am J Med Genet* 1999; **85**: 452.
 48. Garcia-Perez MA, Sanjurjo P, Rubio V. Demonstration of the *spf-ash* mutation in Spanish patients with ornithine transcarbamylase deficiency of moderate severity. *Hum Genet* 1995; **95**: 183.
 49. Hodges PE, Rosenberg LE. The *spf-ash* mouse: a missense mutation in the ornithine transcarbamylase gene also causes aberrant mRNA splicing. *Proc Natl Acad Sci USA* 1989; **86**: 4142.
 50. Garcia-Perez MA, Sanjurjo P, Briones P *et al*. A splicing mutation a nonsense mutation (Y167X) and two missense mutations (I159T and A209V) in Spanish patients with ornithine transcarbamylase deficiency. *Hum Genet* 1995; **96**: 549.
 51. Grompe M, Muzny DM, Caskey CT. Scanning detection of mutations in human ornithine transcarbamylase by chemical mismatch cleavage. *Proc Natl Acad Sci USA* 1989; **86**: 5888.
 52. Riciutti FC, Gelehrter TD, Rosenber LE. X-chromosome inactivation in human liver: confirmation of X-linkage of ornithine transcarbamylase. *Am J Hum Genet* 1976; **28**: 332.
 53. Haan EA, Danks DM, Grimes A, Hoogenraad NJ. Carrier detection in ornithine transcarbamylase deficiency. *J Inher Metab Dis* 1982; **5**: 37.
 54. Hokanson JT, O'Brien WE, Idemoto J, Schafer IA. Carrier detection in ornithine transcarbamylase deficiency. *J Inher Metab Dis* 1978; **93**: 75.
 55. Winter S, Sweetman L, Batshaw ML. Carrier detection in ornithine transcarbamylase deficiency using L-alanine loading test. *Clin Res* 1983; **31**: 112A (Abstr.).
 56. Jakobs C, Sweetman L, Nyhan WL *et al*. Stable isotope dilution analysis of orotic acid and uracil in amniotic fluid. *Clin Chim Acta* 1984; **143**: 1231.
 57. Hauser ER, Finkelstein JE, Valle D, Brusilow SW. Allopurinol-induced orotidinuria: a test for mutations at the ornithine carbamoyl transferase locus in women. *N Engl J Med* 1990; **322**: 1641.
 58. Barshop BA, Nyhan WL, Climent C, Rubio V. Pitfalls in the detection of heterozygosity by allopurinol in a variant form of ornithine transcarbamylase deficiency. *J Inher Metab Dis* 2001; **24**: 513.
 59. Capistrano-Estrada S, Nyhan WL, Marsden DJ *et al*. Histopathological findings in a male with late-onset ornithine transcarbamylase deficiency. *Pediatr Pathol* 1994; **14**: 235.
 60. Maestri NE, Lord C, Glynn M *et al*. The phenotype of ostensibly healthy women who are carriers for ornithine transcarbamylase deficiency. *Medicine* 1998; **77**: 389.
 61. Scaglia F, Zheng Q, O'Brien WE *et al*. An integrated approach to the diagnosis and prospective management of partial ornithine transcarbamylase deficiency. *Pediatrics* 2002; **109**: 150.
 62. Rodeck CH, Patrick AD, Pembrey ME *et al*. Fetal liver biopsy for prenatal diagnosis of ornithine carbamyl transferase deficiency. *Lancet* 1982; **2**: 297.

63. Nussbaum RL, Boggs BA, Beaudet AL *et al.* New mutation and prenatal diagnosis in ornithine transcarbamylase deficiency. *Am J Hum Genet* 1986; **38**: 149.
64. Donn SM, Swartz RD, Thoene JG. Comparison of exchange transfusion peritoneal dialysis and hemodialysis for the treatment of hyperammonemia in an anuric newborn infant. *J Pediatr* 1979; **95**: 67.
65. Batshaw M, MacArthur R, Tuchman M. Alternative pathway therapy for urea cycle disorders: twenty years later. *J Pediatr* 2001; **138**: S46.
66. Pascual JC, Matarredona J, Mut J. Acrodermatitis enteropathica-like dermatosis associated with ornithine transcarbamylase deficiency. *Pediatr Dermatol* 2007; **24**: 394.
67. Deardorff MA, Gaddipati H, Kaplan P *et al.* Complex management of a patient with a contiguous Xp11.4 gene deletion involving ornithine transcarbamylase: a role for detailed molecular analysis in complex presentations of classical disease. *Mol Genet Metab* 2008; **94**: 498.
68. Lee B, Goss J. Long-term correction of urea cycle disorders. *J Pediatr* 2001; **138**: S63.
69. Ensenauer R, Tuchman M, El-Youssef M *et al.* Management and outcome of neonatal-onset ornithine transcarbamylase deficiency following liver transplantation at 60 days of life. *Mol Genet Metab* 2005; **84**: 363.
70. Busuttil AA, Goss JA, Seu P *et al.* The role of orthotopic liver transplantation in the treatment of ornithine transcarbamylase deficiency. *Liver Transplant Surg* 1998; **4**: 350.

Carbamylphosphate synthetase deficiency

Introduction	205	Treatment	207
Clinical abnormalities	205	References	208
Genetics and pathogenesis	207		

MAJOR PHENOTYPIC EXPRESSION

Typical neonatal hyperammonemic crisis, hypocitrullinemia, and absence of activity of hepatic carbamylphosphate synthetase.

INTRODUCTION

Carbamyl phosphate synthetase (CPS) (EC 6.3.4.16) (Figure 25.1) is a mitochondrial enzyme catalyzing the formation of carbamylphosphate from ammonia in what is generally considered to be the first step in the urea cycle. Its deficiency is quite rare, compared with ornithine transcarbamylase deficiency, and usually presents with potentially lethal neonatal hyperammonia [1]. There are even rarer mutations, which led to partial residual activity and a later onset, even adult-onset pattern of disease [2].

Transcription of the gene takes place in the nucleus. The CPS mRNA is found essentially only in the liver, but enzyme activity has been demonstrated in intestinal mucosa. Translation in the cytoplasmic ribosome yields a precursor protein, which then undergoes a complex set of molecular events that eventuate in the appearance of CPS activity in the mitochondrial matrix. The enzyme is a very large dimer with subunits of 160 kDa. The fusion of the two domains joins subunits of striking homology in various species, even including *Escherichia coli* and yeast [3]. An amino terminal leader sequence targets the protein to the mitochondria and is highly basic because of its content of

lysine and arginine residues. Following transport to the mitochondria, this sequence is cleaved to yield the mature protein [4–6]. The CPS enzyme constitutes 15–30 percent of the hepatic mitochondrial protein. A defect at any stage of the sequence from transcription and translocation to transport, uptake, and processing could lead to loss of enzyme activity and clinical disease.

The human cDNA has been cloned and mapped to chromosome 2q35 [7,8]. Mutations reported have included T1370G, and A2429G, which led to V457G and Q810R amino acid substitutions [8,9]. Molecular analysis revealed no immunoreactive enzyme and no translatable mRNA in some patients with lethal neonatal disease [10]. Gross alteration in the gene was not found in the six unrelated families studied [11]. A frequent restriction fragment length polymorphism (RFLP) is a useful genetic marker for linkage analysis in prenatal diagnosis and heterozygote detection [11].

CLINICAL ABNORMALITIES

The clinical abnormalities of CPS deficiency are indistinguishable from those of ornithine transcarbamylase (OTC) deficiency (Chapter 24). In the usual situation, the infant is normal at birth and may do well for a period, usually until feedings begin. Then failure to feed well and lethargy develop. There may be grunting or rapid respiration, hypotonia or hypertonia, convulsions, and hypothermia. This is rapidly progressive to deep coma, in which there is a complete unresponsiveness to stimuli. We have compared this state to

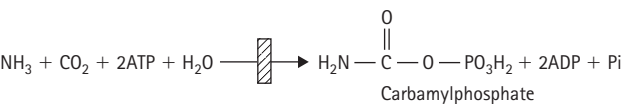


Figure 25.1 The carbamylphosphate synthetase reaction. Acetylglutamate is an obligate activator of the enzyme.

surgical anesthesia. Apnea supervenes and the infant survives only with assisted ventilation. The history often reveals that siblings died very early in life.

A typical history was that of an infant who began to feed poorly, became lethargic, and had convulsions and an abnormal electroencephalogram [12]. She was admitted to hospital at 20 days. Two of her siblings had died with similar symptoms at 4 weeks of age. Despite treatment with a low protein diet, she died at seven months [12, 13]. In similar fashion, a two-month-old Japanese girl [2] had persistent vomiting from 7 days of life, was hypotonic, and had an abnormal electroencephalogram and generalized cerebral cortical atrophy. Another full-term infant appeared normal at birth and was nursed at 10 hours; at 24 hours, he developed profuse sweating and nursed poorly [1]. He had irritability, hypothermia, and hypertonia, along with opisthotonus and ankle clonus. He developed coma and died at 75 hours of age. Of 20 patients reported with this neonatal presentation (outcome was known in 17), all but six died in the neonatal period, and the exceptions died at 5–15 months [1]. An infant with this type of disease is shown in Figure 25.2. Her brother, now mentally impaired, preceded her so she was spared the initial neonatal episode, but she had nearly monthly admissions to hospital for hyperammonemia. This clinical picture is seen in the other urea cycle disorders, which present in the neonatal period. It can be distinguished from the hyperammonemia that occurs in organic acidemias because the serum concentrations of electrolytes do not reveal an acidosis. Blood gases more often indicate a respiratory alkalosis.

A very different type of disorder, the partial deficiency of carbamylphosphate synthetase, was exemplified by a 13-year-old girl [14]. She had episodes of vomiting and lethargy at 3 weeks and 13 months of age, and she

had a transient hemiparesis at two years. She had spastic quadriparesis and severely impaired mental development. A similar patient [15] presented at nine years of age with hyperammonemic coma and was thought to have Reye syndrome. She recovered, but with extensive damage to the brain. She had seizures at 7 days and slow psychomotor development, but was a below average student in a regular school before the episode at nine years. From six years, she had episodic vomiting, abdominal pain, and muscle weakness, lasting 2–3 days. Other patients have had intermittent vomiting and screaming episodes or intermittent lethargy. Impaired mental development has been the rule in those with childhood onset. Seizures and cerebral atrophy on computed tomography (CT) scan are common. We have seen an adult with CPS deficiency who developed his first episode of hyperammonemic coma at the age of 36 years (Figure 25.3). This followed a fugue-like episode for which he had no memory. He gave a history of two previous episodes in which he had periods of a few days for which he had no memory and unexplained scrapes and bruises.

Metabolic stroke has been reported in CPS deficiency [16]. An 18-month-old girl was admitted with somnolence and a left hemiparesis. Magnetic resonance imaging (MRI) revealed infarction of the area supplied by the right middle cerebral artery, but carotid angiography revealed no obstruction of this vessel.

Presentation with coma following the use of valproate to treat seizures was reported in a 32-year-old woman [17]. Onset of agitated disorientation followed by coma and decerebrate posturing and death was reported [18] in a previously asymptomatic woman following pregnancy and delivery.



Figure 25.2 AO: An eight-month-old infant with CPS deficiency. The picture was taken just after recovering from hyperammonemic coma and just prior to a successful liver transplantation.



Figure 25.3 CB: A 38-year old man with CPS deficiency.

The clinical chemistry of CPS deficiency may be unremarkable except for the hyperammonemia, or there may be respiratory alkalosis. Amino acid analysis of the plasma at the time of hyperammonemia reveals elevation in glutamine and usually alanine, and sometimes aspartic acid. In addition, the concentration of lysine may be elevated. The concentration of citrulline is typically low, but values within the normal range have been encountered. Organic acid analysis of the urine is remarkable for the absence of elevations of either orotic acid or uracil. The excretion of 3-methylglutaconic acid may be quite high. Carnitine deficiency has been reported in CPS deficiency [19].

GENETICS AND PATHOGENESIS

CPS deficiency is determined by mutation in an autosomal recessive gene. It occurs in approximately 1 in 60,000 births in the United States.

The molecular defect is in the mitochondrial carbamylphosphate synthetase (EC 6.3.4.16) (Figure 25.1). There are two distinct carbamylphosphate synthetases [20]. The one found exclusively in the cytosol, which has been designated CPS II, is involved in pyrimidine biosynthesis. This enzyme is particularly active in rapidly growing tissues and preferentially utilizes glutamine rather than NH_4^+ , and it is not acetylglutamate dependent. The ammonia-dependent mitochondrial carbamylphosphate synthetase has been designated CPS I. The two CPS enzymes are immunologically distinct [21]. In the mitochondria, CPS I catalyzes the formation of carbamylphosphate from NH_4^+ , HCO_3^- and 2 ATP. Glutamine is not an effective substrate, and acetylglutamate and Mg^{++} are required. X-ray crystallography showed that each ATP is bound at a separate fold in the CPS molecule in the catalysis of the carbamylphosphate product [22]. The two CPS enzymes are located in distinct cellular compartments, but carbamylphosphate, which accumulates in mitochondria when there is a defect in OTC or more distal enzymes, readily makes its way to the cytosol and becomes a substrate for the synthesis of pyrimidines [23], and orotic acid is found in the urine. This is, of course, absent in CPS deficiency. The concentration of urea may be low. Concentrations of citrulline and arginine may be quite low.

In addition to acetylglutamate, carbamylglutamate is an activator of CPS I; glutamate and 2 oxoglutarate are inhibitors [24], as is carbamylphosphate, the product [25].

Deficiency of CPS is readily demonstrable by assay of the enzyme in biopsied liver [1, 26, 27]. It is possible to make the diagnosis of CPS deficiency by assay of the enzyme in biopsied rectal [27] or duodenal [28] tissue. In patients with CPS deficiency, levels of 0–50 percent of normal have been reported [12–14, 26, 28]. In general, the correlation of residual activity with clinical presentation has been good.

Heterozygosity has been documented in a family in which intermediate levels of CPS activity were documented

in biopsied liver of parents [29]. Antenatal diagnosis is possible by assay of the enzyme in biopsied fetal liver or by mutational analysis. The frequent RFLP at the CPS locus found after incubating with BglI is useful in heterozygote detection and prenatal diagnosis. Linkage disequilibrium among four restriction patterns has been found; the A pattern was found in a frequency of 0.83 in affected individuals and 0.20 in controls [30].

The gene (*CPSI*) is enormous. It contains 38 exons [31]. It is located on chromosome 2q35 and spans 122 kb. Most mutations identified have been missense [8, 9, 32, 33], which led to markedly reduced enzyme activity. A homozygous missense mutation was found [33] in a neonatal Japanese patient. In 16 Japanese patients, 25 mutations were identified, 19 of them novel [34]. Mutations common to more than one family were found in 32 percent. Missense mutations clustered around the phosphorylation domains. Nonsense and in/del mutations were widely scattered. In this series, most patients presented in the neonatal period, but a later onset did not ensure a favorable prognosis. One died on presentation at 31 years.

A C+A polymorphism in the gene (p.T1405N) altered the production of nitric oxide, and vascular muscle reactivity and blood flow was greatest in those homozygous for the allele [35].

TREATMENT

The treatment of the acute hyperammonemic crisis of CPS deficiency is as outlined in Chapter 24 [36, 37]. The acute and chronic management of this condition does not differ from that of OTC deficiency. Doses of nabenzoate and naphenylacetate, as well as agininine, begin with 250 mg/kg of each. Maintenance doses of 500 mg/kg of each have been given safely. An antiemetic such as zofran (0.15–0.5 mg/kg) is sometimes useful. For chronic oral treatment, naphenylbutyrate has been employed in doses of 250–600 mg/kg per day. Citrulline is used in doses of 150–600 mg/kg. The dietary intake of protein is restricted and supplementation with a mixture of essential amino acids (cyclinex, EAMI, UCD) is usually helpful in maintaining reasonable concentrations of amino acids in plasma while minimizing nitrogen load.

N-carbamylglutamate is an analog of N-acetylglutamate which activates carbamylphosphate synthetase [38]. The advantage is that it enters mitochondria and is not hydrolyzed by cytosolic deacylases, as is acetylglutamate. The compound, at 100–300 mg/kg has been reported to be effective in the management of acetylglutamate synthetase (AGS) deficiency [39, 40]. Doses of 300–1800 mg/day have been well tolerated, and long-term management has been reported. The compound had been suggested [40] as a test for AGS deficiency, which might distinguish it from carbamylphosphate synthetase deficiency. However, the compound has now been reported to be quite effective in the management of a patient with CPS deficiency [41].

REFERENCES

- Gelehrter TD, Snodgrass PJ. Lethal neonatal deficiency of carbamylphosphate synthetase. *N Engl J Med* 1974; **290**: 430.
- Arashima S, Matsuda I. A case of carbamylphosphate synthetase deficiency. *Tohoku J Exp Med* 1972; **107**: 143.
- Nyunoya H, Broglie KE, Widgren EE, Lusty CJ. Characterization and derivation of the gene coding for mitochondrial carbamylphosphate synthetase I of rat. *J Biol Chem* 1985; **75**: 5071.
- Mori M, Miura S, Tatibana M, Cohen PP. Cell-free synthesis and processing of a putative precursor for mitochondrial carbamylphosphate synthetase I of rat liver. *J Biol Chem* 1979; **76**: 5071.
- Shore GL, Carignan P, Raymond Y. *In vitro* synthesis of a putative precursor to the mitochondrial enzyme carbamylphosphate synthetase. *J Biol Chem* 1979; **254**: 3141.
- Raymond Y, Shore GL. The precursor for carbamylphosphate synthetase is transported to mitochondria via a cytosolic route. *J Biol Chem* 1979; **254**: 9335.
- Adcock MW, O'Brien WE. Molecular cloning of cDNA for rat and human carbamyl phosphate synthetase I. *J Biol Chem* 1984; **259**: 13471.
- Funghini S, Donati MA, Pasquini E *et al*. Molecular studies of CPS1 gene: determination of genomic organization and mutation detection. *Am J Hum Genet* 2000; **66**: 418.
- Funghini S, Donati MA, Pasquini E *et al*. Genomic organization of human CPS1 gene and identification of two new mutations in a CPSD patient. *J Inherit Metab Dis* 2002; **25**(Suppl. 1): 052P.
- Graf L, McIntyre P, Hoogenraad N. A carbamylphosphate synthetase deficiency with no detectable immunoreactive enzyme and no translatable mRNA. *J Inherit Metab Dis* 1984; **7**: 104.
- Fearon ER, Mallonee RL, Philipps JA III *et al*. Genetic analysis of carbamylphosphate synthetase 1 deficiency. *Hum Genet* 1985; **70**: 207.
- Hommes FA, DeGroot CJ, Wilmink CW, Jonxis JHP. Carbamylphosphate synthetase deficiency in an infant with severe cerebral damage. *Arch Dis Child* 1969; **44**: 688.
- Ebels EJ. Neuropathological observations in a patient with carbamylphosphate synthetase deficiency and in two sibs. *Arch Dis Child* 1972; **47**: 47.
- Batshaw M, Brusilow S, Walser M. Treatment of carbamyl phosphate synthetase deficiency with keto analogues of essential amino acids. *N Engl J Med* 1985; **292**: 1085.
- Granot E, Matoth I, Lotan C *et al*. Partial carbamylphosphate synthetase deficiency, simulating Reye's syndrome, in a 9-year-old girl. *Isr J Med Sci* 1986; **22**: 463.
- Sperl W, Felber S, Skladal D, Wemuth B. Metabolic stroke as a novel observation in carbamylphosphate synthetase deficiency. *Proc SSIEM* 1995: P036.
- Verbiest HBC, Straver JS, Colombo JP *et al*. Carbamylphosphate synthetase-1 deficiency discovered after valproic acid-induced coma. *Acta Neurol Scand* 1992; **86**: 275.
- Wong L-JC, Craigen WJ, O'Brien WE. Postpartum coma and death due to carbamoyl-phosphate synthetase I deficiency. *Ann Intern Med* 1994; **120**: 216.
- Mori T, Tsuchiyama A, Nagai K *et al*. A case of carbamylphosphate synthetase-I deficiency associated with secondary carnitine deficiency-L carnitine treatment of CPS-I deficiency. *Eur J Pediatr* 1990; **149**: 272.
- Tatibana M, Ito K. Control of pyrimidine biosynthesis in mammalian tissues. I. Partial purification characterization of glutamine-utilizing carbamylphosphate synthetase of mouse spleen and its tissue distribution. *J Biol Chem* 1969; **244**: 5403.
- Nakanishi S, Ito K, Tatibana M. Two types of carbamylphosphate synthetase in rat liver: chromatographic resolution and immunological distinction. *Biochem Biophys Res Commun* 1968; **33**: 774.
- Kothe M, Purcarea C, Hedeel GI *et al*. Direct demonstration of carbamoylphosphate formation on the C-terminal domain of carbamoylphosphate synthetase. *Protein Sci* 2005; **14**: 37.
- Natale PJ, Tremblay GC. On the availability of intramitochondrial carbamylphosphate for the extramitochondrial biosynthesis of pyrimidines. *Biochem Biophys Res Commun* 1969; **37**: 512.
- Marshall M, Metzberg RL, Cohen PP. Physical and kinetic properties of carbamylphosphate synthetase from frog liver. *J Biol Chem* 1961; **236**: 2229.
- Elliot KRF. Kinetic studies on mammalian liver carbamylphosphate synthetase. In: Grisolia S, Baguena R, Mayor F (eds). *The Urea Cycle*. New York: John Wiley & Sons, 1976: 123.
- Odievre C, Charpentier C, Cathelineau L *et al*. Hyperammoniemie constitutionnelle avec deficit en carbamyl-phosphate-synthetase. Evolution sous regime dietetique. *Arch Fr Pediatr* 1973; **30**: 5.
- Hoogenraad NJ, Mitchell JD, Don NA *et al*. Detection of carbamyl phosphate synthetase I deficiency using duodenal biopsy samples. *Arch Dis Child* 1980; **55**: 292.
- Matsushima A, Orii T. The activity of carbamylphosphate synthetase I (CPS I) and ornithine transcarbamylase (OTC) in the intestine and the screening of OTC deficiency in the rectal mucosa. *J Inherit Metab Dis* 1981; **4**: 83.
- McReynolds JW, Crowley B, Mahoney MJ, Rosenberg LE. Autosomal recessive inheritance of human mitochondrial carbamyl phosphate synthetase deficiency. *Am J Hum Genet* 1981; **33**: 345.
- Malonee R, Fearon E, Philipps J III *et al*. Genetic analysis of carbamyl-phosphate synthesis I deficiency. *Hum Genet* 1985; **70**: 207.
- Summar ML, Hall LD, Eeds AM *et al*. Characterization of genomic structure and polymorphisms in human carbamylphosphate synthetase I gene. *Gene* 2003; **311**: 51.
- Guillou F, Liao M, Garcia-Espana A, Lusty CJ. Mutational analysis of carbamylphosphate synthetase. Substitution of Glu841 leads to loss of functional coupling between the two catalytic domains of the synthetase subunit. *Biochemistry* 1992; **31**: 1656.
- Hoshida R, Matsuura T, Haraguchi Y *et al*. Carbamylphosphate synthetase I deficiency; one base substitution in an exon of the CPS I gene causes a 9-base pair deletion due to aberrant splicing. *J Clin Invest* 1993; **91**: 1884.

34. Kurokawa K, Yorifuji T, Kawai M *et al.* Molecular and clinical analyses of Japanese patients with carbamoylphosphate synthetase 1 (CPS1) deficiency. *J Hum Genet* 2007; **52**: 349.
35. Summar ML, Gainer JV, Pretorius M *et al.* Relationship between carbamoyl-phosphate synthetase genotype and systemic vascular function. *Hypertension* 2004; **43**: 186.
36. Batshaw ML, Brusilow S, Waber L *et al.* Treatment of inborn errors of urea synthesis; activation of alternative pathways of waste nitrogen synthesis and excretion. *N Engl J Med* 1982; **306**: 1387.
37. Brusilow WS, Danney M, Waber LJ *et al.* Treatment of episodic hyperammonemia in children with inborn errors of urea synthesis. *N Engl J Med* 1984; **310**: 1630.
38. Grisolia S, Cohen PP. The catalytic role of carbamylglutamate in citrulline biosynthesis. *J Biol Chem* 1952; **198**: 561.
39. Bachmann C, Colombo JP, Jaggi K. N-Acetylglutamate synthetase (NAGS) deficiency: diagnosis, clinical observations and treatment. In: Lowenthal A, Mori A, Marescau B (eds). *Urea Cycle Diseases*. Advances in Experimental Medicine and Biology, vol. 153. New York: Plenum Press, 1982: 39.
40. Rubio V, Grisolia S. Treating urea cycle defects. *Nature* 1981; **292**: 496.
41. Kuchler G, Rabier D, Poggi-Travert F *et al.* Therapeutic use of carbamylglutamate in the case of carbamylphosphate synthetase deficiency. *Proc SSIEM* 1995: P037.

Citrullinemia

Introduction	210	Treatment	213
Clinical abnormalities	210	References	214
Genetics and pathogenesis	212		

MAJOR PHENOTYPIC EXPRESSION

Potentially lethal coma; convulsions; hyperammonemia; hypercitrullinemia; orotic aciduria; and defective activity of argininosuccinate synthetase.

INTRODUCTION

Citrullinemia was first reported in 1963 [1] in a patient with impaired mental development. Soon after, it became apparent that the classic presentation is as a typical neonatal hyperammonemia that was, until the development of modern methods of pharmacologic therapy, uniformly lethal [2–8]. The picture is indistinguishable from that of the male neonate with ornithine transcarbamylase deficiency (Chapter 24). The activity of argininosuccinate synthetase (EC 6.3.4.5) is widely expressed in tissues (Figure 26.1). Its deficiency is readily demonstrated in cultured fibroblasts [9]. The gene (*CTNLI*) has been cloned [10] and mapped to chromosome 9 at q34 [11]. A number and variety of mutations have been described [12–15].

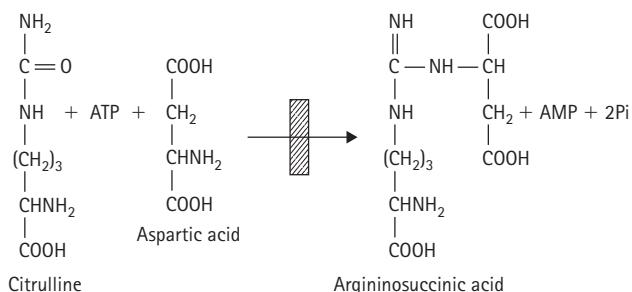


Figure 26.1 The argininosuccinic acid synthetase reaction, site of the defect in citrullinemia.

CLINICAL ABNORMALITIES

Citrullinemia usually presents as an overwhelming neonatal illness. Following a brief hiatus in which the newborn appears normal, anorexia, vomiting, and lethargy develop, and these symptoms are followed rapidly by progression to



Figure 26.2 JPN: A 12-day-old infant with citrullinemia, illustrating deep coma requiring assisted ventilation. The concentration of ammonia in plasma was 770 $\mu\text{mol/L}$. He was flaccid and completely unresponsive. He had required assisted ventilation, but this was discontinued after a series of exchange transfusions that temporarily lowered the ammonia to 236 $\mu\text{mol/L}$. Concentrations of ammonia were over 1000 mg/dL at 4 hours.

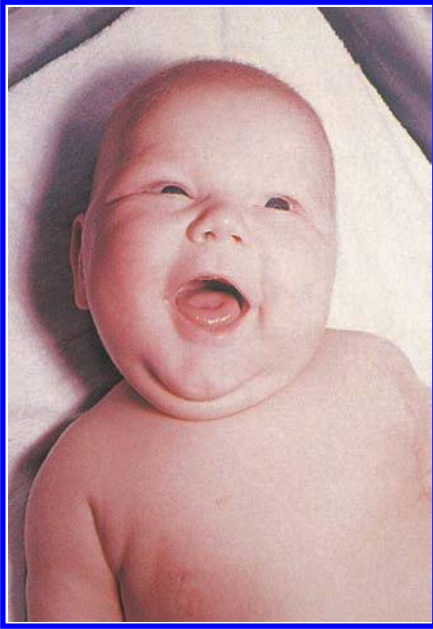


Figure 26.3 JPN: Two months after the time of Figure 26.2. Treated with keto acid analogs of amino acids, he had recovered well and was alert and appeared to be developing normally. He was rather chubby. He died before his first birthday of acute hyperammonemic coma.



Figure 26.5 RM: Four months later. He was rather obese and had frequent diaper rashes consistent with protein inadequacy. In follow up, he had mildly impaired mental development despite having had only two subsequent slightly hyperammonemic attacks.



Figure 26.4 RM: A newborn with citrullinemia. He developed hyperammonemic coma at 3 days of age. He was treated with exchange transfusion, arginine, and nabenzolate/phenylacetate. The hyperammonemia resolved, but the level of citrulline in plasma was more than 1000 $\mu\text{mol/L}$.



Figure 26.6 NF: At 4 days of age. She appeared to be normal and had a normal level of ammonia, electroencephalogram, and computed tomography scan. Treatment was initiated at birth. Her sister had died of citrullinemia in the neonatal period. A prenatal diagnosis permitted treatment of NF from birth, which prevented neonatal hyperammonemia. The concentration of citrulline in the amniotic fluid at mid-gestation was 80 $\mu\text{mol/L}$ (1.4 g/dL) (normal range, 0–23 mmol/L) and the activity of the enzyme was 8 percent of the control mean. She had many other episodes of acute hyperammonemia and they seemed to become more frequent and more worrisome during teenagehood. A liver transplantation at the age of 16 years led to a cessation of these episodes.

deep coma (Figures 26.2, 26.3, 26.4, 26.5, 26.6, and 26.7). Apnea ensues and death is inevitable, unless the infant is intubated and provided with mechanical ventilation. Seizures often occur and there are abnormalities of the electroencephalogram (EEG). The infant may have hypertonia and there may be decerebrate posturing. The neurologic abnormality is progressive to flaccidity and dilated, fixed pupils. The infant is unresponsive even to deep pain. The liver may be enlarged and serum levels of transaminases are often elevated.

Citrullinemia is genetically heterogeneous and there have been a variety of different clinical pictures in patients with partial residual activity of the defective enzyme. All of these variants are encountered less frequently than the classic infantile one. Some of these patients have had a more gradual onset of difficulty with feedings and recurrent or cyclic vomiting in infancy. Some have had hepatomegaly and elevation of the serum glutamate-



Figure 26.7 VT: An infant with citrullinemia who appeared normal at two months. A previous sibling had died of overwhelming neonatal citrullinemia.

oxaloacetate transaminase (SGOT) or serum glutamate pyruvate transaminase (SGPT), which may cause confusion by suggesting a diagnosis of hepatocellular disease [6]. This is true also of the classic acute infantile disease [16]. The prothrombin and partial thromboplastin time may be prolonged. Cirrhosis at 17 months of age has been reported [17]. Acute hepatic failure which led to referral for liver transplantation occurred in two infants [18] in whom medical treatment reversed the hepatic changes. Episodic hyperammonemia may occur with vomiting, lethargy, headaches, tremors, seizures, or ataxia [19]. Some degree of impaired mental development is usually present [1], and computed tomography (CT) or magnetic resonance imaging (MRI) scan usually reveals evidence of cerebral atrophy.

A late onset form of citrullinemia is especially common in Japan [20]. It is now clear that most of these patients have had citrin deficiency (OMIM 603472) in which the mutations are in the *SLC25A13* gene [21]. Additionally, patients have been seen in the United States, Israel, China, Korea, and Vietnam, many with the same mutations found in Japanese. We have also seen variant, attenuated presentations in patients with mutations in the *CTNLI1* gene. Cravings have been reported for foods such as beans, peas, and peanuts, which are rich in arginine [22].

One patient in whom citrullinemia was found on routine screening had had no clinical evidence of disease at the time of report [3, 23]. Even the patient with a variant form of citrullinemia, in whom symptomatology has been mild, or even absent for long periods, may become neurologically incapacitated in childhood or adulthood. Patients with this disease have had strokes (Appendix) [24].

The most prominent metabolic characteristic of citrullinemia is the hyperammonemia, which is usually massive in the neonatal form, but deep coma mimicking anesthesia has been seen with concentrations of 400 mmol/L [16]. Concentrations in the blood of glutamine and usually of alanine are also elevated, and

often that of aspartic acid as well. The concentration of arginine is usually decreased. Excretion of orotic acid is increased [25], although usually not to the degree seen in ornithine transcarbamylase deficiency.

Concentrations of citrulline in body fluids of these patients are very high. Plasma concentrations usually approximate 40 times normal; levels of 850–4600 $\mu\text{mol/L}$ are commonly encountered. The lowest level reported of 290 $\mu\text{mol/L}$ was found in a patient who had no clinical evidence of disease [3]. Urinary excretion of citrulline may range from several hundred milligrams per day in an infant to several grams per day in an older patient. Some patients may also excrete homocitrulline, homoarginine, or N-acetylcitrulline [1, 2, 6, 18, 19, 26]. Concentrations of citrulline are also elevated in the cerebrospinal fluid, but levels are lower than in the blood.

GENETICS AND PATHOGENESIS

Citrullinemia is transmitted as an autosomal recessive disease. Intermediate levels of activity of argininosuccinate synthetase have been found in fibroblasts of parents [7–9, 16]. Prenatal diagnosis has been made by assay of the concentration of citrulline in amniotic fluid or by assay of the enzyme in cultured amniocytes (Figure 26.6) [4, 16]. Prenatal diagnosis may also be accomplished by assay of the enzyme in chorionic villus material [27], but very low levels of enzyme in heterozygotes may be a real problem. For this reason, a sensitive radiochemical assay was developed. The most reliable approach is to assess the enzyme in cultured amniocytes. Restriction fragment length polymorphism (RFLP) may permit detection of heterozygotes and prenatal diagnosis, and the existence of three distinct RFLPs [28], as well as a highly polymorphic variable number tandem repeat (VNTR) [29], increases the likelihood that linkage analysis may be useful in an individual family.

The molecular defect in citrullinemia is in the enzyme, argininosuccinic acid synthetase. This is a cytosolic enzyme in contrast to ornithine transcarbamylase and carbamylphosphate synthetase (Chapters 25 and 26). Argininosuccinic acid synthetase catalyzes the conversion of citrulline and aspartic acid to argininosuccinic acid (Figure 26.1). The enzyme is widely distributed in tissues. The defect has usually been demonstrated in cultured fibroblasts [8, 9] and it has also been demonstrated in liver [1, 4, 19]. Activity in the neonatal form of the disease is usually virtually zero.

In a patient with a variant form of citrullinemia, 5 percent of normal activity was found in liver [30]. In the adult Japanese phenotype, levels as high as 50 percent of control have been reported [20]. Kinetic studies have revealed K_m values for citrulline as high as 200 times normal in variants with alterations in the structure of the enzyme protein [9, 31]. In one patient, the activity of the enzyme in brain was lower than that found in the liver [32]. In the adult onset Japanese phenotype, the deficiency

is specific to the liver [33] and not found in other tissues. These patients have normal levels of hepatic mRNA, but a decreased amount of enzyme-linked immunosorbent assay (ELISA) detectable enzyme protein [34].

Cloning of the cDNA for argininosuccinic acid synthetase was facilitated by the use of a cultured human cell line in which very high levels of mRNA for this enzyme were produced when the cells were cultivated in medium containing canavanine, an analog of arginine [10]. The gene was sequenced and found to contain 63 kb in 16 exons [11]. It codes for a monomeric protein of 46 kDa that forms a tetramer [35]. Expression of the gene is highly regulated, increasing with fasting, dexamethasone, or dibutyryl-cAMP, and the substitution of citrulline for arginine in medium, and decreasing in response to arginine.

Analysis of the DNA of 11 patients by Southern blot failed to reveal major rearrangements of the gene [36]. Analysis of the mRNA and protein of these lines revealed considerable heterogeneity. Nine of 11 were devoid of cross-reacting material (CRM), while they had all mRNA. Levels of CRM and enzyme activity below 50 percent were found in some parents. Among patients with classic citrullinemia at least 20 mutations have been reported [37],

indicating a considerable heterogeneity. Most patients are compound heterozygotes.

An interesting homozygous mutation found in the child of consanguineous parents was a G-to-C substitution in the splice acceptor site of the terminal intron, which abolished normal splicing and led to three abnormal splice products, the most common of which was translated to a protein 25 amino acids longer, but so unstable that CRM was not detectable [12]. Most of the other alterations found have been missense mutations (Table 26.1) [13, 37]. A number have involved CpG dinucleotides. Deletions of entire exons have resulted from deletion of genomic sequences [14]. Nonsense mutations were until recently not reported, but a nonsense mutation in codon 86 (arginine) was found in citrullinemic cattle [15]. The missense mutations found in classic neonatal citrullinemia have all altered an amino acid that was highly conserved, most of them across eight species ranging from humans to *Saccharomyces* and *Escherichia coli* [37]. Elucidation of the genomic sequence of the gene has permitted the use of intronic primers and has simplified mutational analysis [38]. Novel mutations p.W179R and p.G326V have been found in patients with attenuated or asymptomatic citrullinemia [38]. A summary of 87 mutations [39], 27 novel, indicated distribution through exons 13 to 15, but with most in exons 15, 12, 13, and 14. G390R was the most frequent in patients with the classic phenotype. In a patient in whom no mRNA could be detected, a transposition at 279 converted an arginine to a stop codon [40]. It was concluded that the RNA negative phenotype resulted from nonsense-mediated mRNA decay.

Table 26.1 Mutations defined in classic citrullinemia

Mutation	Deletions, insertions	Exon
G14S		3
S18L		4
R86C	Del Exon 5	5
A118T		6
	Del Exon 6	6
	IVS6-2	
R157H	Del Exon 7	7
S180N		8
A192V		9
		10
		11
R272P		12
R279Q		
G280R		
R304W	Del Exon 13	13
G324S		
R363W		14
R363L	IVS15-1	
G390R	Insertion 37 b, exons 15, 16	15
	Del 76 Exon 16	16

b, bases; Del, deletion.

A, alanine; C, cysteine; G, glycine; H, histidine; L, leucine; N, asparagine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan.

TREATMENT

The acute management of the initial neonatal hyperammonemia and subsequent intercurrent attacks is set out in Chapter 24, along with general principles of long-term management of hyperammonemic infants using sodium benzoate and/or phenylacetate and arginine. The infant with citrullinemia differs in that even an acute crisis of hyperammonemia can be managed with intravenous arginine alone, as long as the episode is treated promptly and the level of ammonia is not too high. It would not be recommended to treat the initial infantile

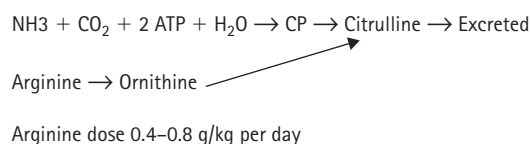


Figure 26.8 Chronic management of citrullinemia. Of the three N atoms of citrulline, two come from ornithine administered as arginine. Hence, a net of one N is lost for every molecule of citrulline excreted. As long as ornithine is supplied, this minicycle continues to operate. CP, carbamylphosphate.

crisis with anything less than a full-scale attack on the hyperammonemia. In this disease, whether benzoate and/or phenylacetate is employed, it is well to employ doses of arginine in both the priming and sustaining infusion of at least 0.66 g/kg.

Long-term steady-state management can usually be provided with arginine and a diet modestly restricted in protein (Figure 26.8) [16, 41]. The principle is that if ornithine molecules can be provided through supplemental arginine, in order to keep the urea cycle operating, waste nitrogen can be adequately eliminated in the form of citrulline. Citrulline contains only one more nitrogen atom than the two provided as ornithine. It is probably not as efficient as urea in eliminating nitrogen, but this is sufficient except at times of catabolism. If the patient is vomiting or for some other reason cannot take oral arginine, hospital admission for intravenous arginine therapy is mandatory. Oral doses of arginine employed have ranged from 0.25 to 0.8 g/kg per day. Normal development has been reported with such a regimen [41]. We have observed low levels of essential amino acids, especially of branched amino acids, in patients treated with benzoate, phenylacetate, or phenylbutyrate. This has also been reported [42]. There may be clinical signs of protein inadequacy (Figures 26.5 and 26.9). Supplementation with mixtures of essential amino acids is preferable to increasing whole protein in this instance, and nitrogen-free analogs of amino acids are useful [43], but these mixtures are no longer available. This problem of essential amino acid depletion has not been observed with arginine treatment.

Prognosis for intellectual development probably



Figure 26.9 NF: Her very short hair illustrates the fact that among patients with urea cycle defects, the loss of hair has been our most sensitive index of protein inadequacy.

depends on the nature of the initial hyperammonemia, especially its duration [44] or those of recurrent episodes. The ability to prevent hyperammonemia by early treatment in patients diagnosed prenatally [45] should be consistent with a better prognosis, but only if recurrent attacks of hyperammonemia are prevented or treated early and effectively. In the most recent assessment of the collaborative study managed by Brusilow and colleagues [42], 24 patients had a five-year survival of 87.5 percent and a ten-year survival of 72 percent, indicating the always dangerous nature of this disease. Of 15 survivors, 11 were severely or profoundly mentally impaired, and in four IQ values were in the borderline range. Growth in many was impaired, but most had height-for-weight scores within 2 SD of the mean.

REFERENCES

1. McMurray WC, Rathbun JC, Mohyuddin F *et al.* Citrullinemia. *Pediatrics* 1963; **32**: 347.
2. Vander Zee SPM, Trijbels JMF, Monnens LAH *et al.* Citrullinaemia with a rapidly fatal neonatal course. *Arch Dis Child* 1971; **48**: 847.
3. Ghisolfi J, Augier D, Martinez J *et al.* Forme neonatale de citrullinemia a l'evolution mortelle rapide. *Pediatric* 1972; **27**: 55.
4. Wick H, Bachmann C, Baumgartner R *et al.* Variants of citrullinaemia. *Arch Dis Child* 1973; **48**: 636.
5. Roerdink FH, Gouw WL, Okken A *et al.* Citrullinemia. Report of a case with studies on antenatal diagnosis. *Pediatr Res* 1973; **7**: 863.
6. Danks DM, Tipett P, Zenter G. Severe neonatal citrullinemia. *Arch Dis Child* 1974; **49**: 579.
7. Buist NRM, Kennaway NG, Hepburn CA *et al.* Citrullinemia: investigation and treatment over a four-year period. *J Pediatr* 1974; **85**: 208.
8. Leibowitz J, Thoene J, Spector E, Nyhan WL. Citrullinemia. *Virch Arch A Pathol Anat Histol* 1978; **377**: 249.
9. Kennaway NG, Harwood PJ, Ramberg DA *et al.* Citrullinemia: enzymatic evidence for genetic heterogeneity. *Pediatr Res* 1975; **9**: 554.
10. Su TS, Bock HGO, O'Brien WE, Beaudet AL. Cloning of cDNA for arginino-succinate synthetase mRNA and study of enzyme over-production in a human cell line. *J Biol Chem* 1981; **256**: 11826.
11. Su TS, Nussbaum RL, Airpart S *et al.* Human chromosomal assignments for 14 argininosuccinate synthetase pseudogenes: cloned DNAs as reagents for cytogenetic analysis. *Am J Hum Genet* 1984; **36**: 954.
12. Su TS, Lin LH. Analysis of splice acceptor site mutation which produces multiple splicing abnormalities in the human argininosuccinate synthetase locus. *J Biol Chem* 1990; **265**: 19716.
13. Kobayashi K, Jackson M, Tick DB, O'Brien WE. Heterogeneity of mutations in argininosuccinate synthetase causing human citrullinemia. *J Biol Chem* 1990; **265**: 11361.

14. Jackson MJ, Allen SJ, Beaudet AL, O'Brien WE. Metabolite regulation of argininosuccinate synthetase in cultured human cells. *J Biol Chem* 1988; **263**: 16388.
15. Dennis JA, Healy PJ, Beaudet AL, O'Brien WE. Molecular definition of bovine argininosuccinate synthetase deficiency. *Proc Natl Acad Sci USA* 1989; **86**: 7947.
16. Nyhan WL, Sakati NA. Citrullinemia. In: Nyhan WL, Sakati NA (eds). *Diagnostic Recognition of Genetic Disease*. Philadelphia: Lea and Febiger, 1987: 159.
17. Gucer S, Asan E, Atilla P *et al*. Early cirrhosis in a patient with type I citrullinaemia (CTLN1). *J Inherit Metab Dis* 2004; **27**: 541.
18. De Groot MJ, Cuppen M, Eling M *et al*. Metabolic investigations prevent liver transplantation in two young children with citrullinemia type I. *J Inherit Metab Dis* September 8, 2010 [Epub ahead of print].
19. Morrow G III, Barness LA, Efron ML. Citrullinemia with defective urea production. *Pediatrics* 1967; **40**: 565.
20. Kooka T, Higashi Y, Uebayashi Y, Kobayashi R. A special form of hepatocerebral degeneration with citrullinemia. *Neurol Med* 1977; **6**: 47.
21. Lu YB, Kobayashi K, Ushikai M *et al*. Frequency and distribution in east Asia of 12 mutations identified in the SLC25A13 gene of Japanese patients with citrin deficiency. *J Hum Genet* 2005; **50**: 338.
22. Paul AA, Southgate DAT. *McCance and Widdowson's The Composition of Foods*, 4th edn. New York: Elsevier/ North-Holland, Biomedical Press, 1978.
23. Wick H, Brechbühler T, Girard J. Citrullinemia: elevated serum citrulline levels in healthy siblings. *Experientia* 1970; **26**: 823.
24. Testai FD, Gorelick PB. Inherited metabolic disorders and stroke part 2: homocystinuria, organic acidurias, and urea cycle disorders. *Arch Neurol* 2010; **67**: 148.
25. Bachmann C. Urea cycle. In: Nyhan WL (ed.). *Heritable Disorders of Amino Acid Metabolism*. New York: John Wiley & Sons, 1974: 361.
26. Strandholm JJ, Buist NRM, Kennaway NG, Curtis HT. Excretion of N-acetyl-citrulline in citrullinemia. *Biochim Biophys Acta* 1971; **244**: 214.
27. Fleisher L, Mitchell D, Koppitch F *et al*. Chorionic villus samples (CVS) for the prenatal diagnosis of aminoacidopathies. *Am J Hum Genet* 1984; **36**: 188S.
28. Northrup H, Lathrop M, Lu SY *et al*. Multilocus linkage analysis with the human argininosuccinate synthetase gene. *Genomics* 1989; **5**: 442.
29. Kwiatowski DJ, Nygaard TG, Schuback DE *et al*. Identification of a highly polymorphic microsatellite VNTR within the argininosuccinate locus: exclusion of the dystonia gene on 9q32-34 as the cause of dopa-responsive dystonia in a large kindred. *Am J Hum Genet* 1991; **48**: 121.
30. McMurray WC, Mohyuddin F, Bayer SM, Rathbun JD. Citrullinuria: a disorder of amino acid metabolism associated with mental retardation. In: Oster J, Sletved HV (eds). *Proceedings of International Copenhagen Congress on the Scientific Study of Mental Retardation*. Copenhagen: Det Berlinske Bogtrykkeri, 1965: 117.
31. Tedesco TA, Mellman WJ. Argininosuccinate synthetase activity and citrulline metabolism in cells cultured from a citrullinemic subject. *Proc Natl Acad Sci USA* 1967; **57**: 169.
32. Christensen E, Brandt NJ, Philip J *et al*. Citrullinaemia: the possibility of prenatal diagnosis. *J Inherit Metab Dis* 1980; **3**: 73.
33. Saheki T, Kobayashi K, Inoue I. Hereditary disorders of the urea cycle in man: biochemical and molecular approaches. *Rev Physiol Biochem Pharmacol* 1987; **108**: 21.
34. Saheki T, Kobayashi K, Ichiki H *et al*. Molecular basis of enzyme abnormalities in urea cycle disorders. In *Recent Advances in Inborn Errors of Metabolism*. Proceedings of 4th International Congress. *Enzyme* 1987; **38**: 227.
35. Bock HGO, Su TS, O'Brien WE, Beaudet AL. Sequences for human argininosuccinate synthetase cDNA. *Nucleic Acids Res* 1983; **11**: 6505.
36. Su TS, Bock HQ, Beaudet AL, O'Brien WE. Molecular analysis of argininosuccinate synthetase deficiency in human fibroblasts. *J Clin Invest* 1982; **70**: 1334.
37. Kobayashi K, Shaheen N, Terazono H, Saheki T. Mutations in argininosuccinate synthetase mRNA of Japanese patients causing classic citrullinemia. *Am J Hum Genet* 1994; **55**: 1103.
38. Haberle J, Pauli S, Linnebank M *et al*. Structure of the human argininosuccinate synthetase gene and an improved system for molecular diagnostics in patients with classical and mild citrullinaemia. *Hum Genet* 2002; **4**: 327.
39. Engel K, Hohne W, Haberle J. Mutations and polymorphisms in the human argininosuccinate synthetase (ASS1) gene. *Hum Mutat* 2009; **3**: 300.
40. Li CM, Chao HK, Liu YF *et al*. A nonsense mutation is responsible for the RNA-negative phenotype in human citrullinaemia. *Eur J Hum Genet* 2001; **9**: 685.
41. Melnyk AR, Matalon R, Henry BW *et al*. Prospective management of a child with neonatal citrullinemia. *J Pediatr* 1993; **122**: 96.
42. Maestri NE, Clissold DB, Brusilow SW. Long-term survival of patients with argininosuccinate synthetase deficiency. *J Pediatr* 1995; **127**: 929.
43. Thoene J, Batshaw M, Spector E *et al*. Neonatal citrullinemia: treatment with ketoanalogues of essential amino acids. *J Pediatr* 1977; **90**: 218.
44. Msall M, Batshaw ML, Suss R *et al*. Neurologic outcome in children with inborn errors of urea synthesis. *N Engl J Med* 1984; **301**: 1500.
45. Donn SM, Wilson GN, Thoene JG. Prevention of neonatal hyperammonemia in citrullinemia. *Clin Res* 1984; **32**: 806A.

Argininosuccinic aciduria

Introduction	216	Treatment	220
Clinical abnormalities	216	References	220
Genetics and pathogenesis	219		

MAJOR PHENOTYPIC EXPRESSION

Hyperammonemia leading to lethargy and coma; convulsions; hepatic fibrosis, hypertension; hyperglutaminemia and hyperalaninemia; argininosuccinic aciduria and defective activity of argininosuccinate lyase.

INTRODUCTION

Argininosuccinic aciduria was first recognized in patients with chronic more indolent disease where the major manifestations were nonspecific, sometimes mild or moderate mental impairment [1–4]. This may reflect the unique features of the hair in this disorder, which brought many of the early patients to attention with apparent alopecia. The disorder presents also, and probably more frequently, in the classic neonatal hyperammonemic pattern of a typical urea cycle disease [5–10]. Sometimes these infants may be suspected clinically to be different from those with other urea cycle disorders because of the magnitude of the hepatomegaly.

The enzyme argininosuccinate lyase, or argininosuccinase (EC 4.3.2.1) (Figure 27.1), catalyzes the conversion

of the argininosuccinate formed from citrulline and aspartate, to fumarate and arginine, the last compound of the urea cycle prior to the urea splitting off. The cDNA for the human gene has been cloned [11] and the gene has been localized to chromosome 7cen-q11.2 [12]. Mutations have been defined [13–15], some of which have led to alternative splicing.

CLINICAL ABNORMALITIES

The classic presentation of argininosuccinic aciduria is as overwhelming illness in the newborn period. Prior to the development of modern methods of pharmacologic therapy, the end result of this presentation was uniformly fatal. The picture is indistinguishable from that of the male infant with ornithine transcarbamylase deficiency (Chapters 24 and 25). Following a brief hiatus in which the newborn appears normal, anorexia or vomiting and lethargy develop, and these symptoms are rapidly progressive to deep coma, apnea, and death, unless the baby is intubated and maintained via mechanical ventilation. Seizures often occur, along with abnormalities of the electroencephalogram (EEG). The infant may have hypertonia or hypotonic or there may be decerebrate posturing. This condition is progressive to flaccidity and dilated fixed pupils. The infant is unresponsive even to deep pain. There may be hypothermia. Patients may have tachypnea and respiratory alkalosis, which are general consequences of hyperammonemia [16]. A bulging fontanel usually indicates the presence of cerebral edema, but

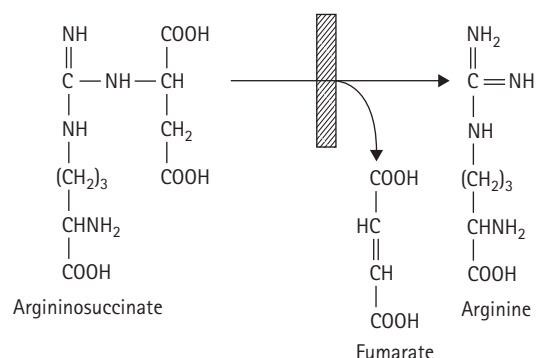


Figure 27.1 The reaction catalyzed by argininosuccinase.

cerebral hemorrhages have been seen in hyperammonemic infants, as have fatal pulmonary hemorrhages.

As in the case of other disorders of the urea cycle (Chapter 24 (ornithine transcarbamylase deficiency), Chapter 25 (carbamylphosphate synthetase deficiency), Chapter 26 (citrullinemia)), argininosuccinic aciduria is genetically heterogeneous and patients with variant forms of the enzyme in which there is partial residual activity may have more indolent forms of the disease [17]. Such patients may present simply with impaired mental development [2, 4] or a convulsive disorder. Commonly, there is episodic disease, such as cyclic vomiting or recurrent headache, ataxia, tremulousness, or lethargy. The classic, and most common phenotype is the neonatal form, with rapid progression to coma in the first days of life. In a subacute or late onset type, the disease becomes manifest in late infancy or childhood. They may survive with impaired mental development, intermittent ataxia, or seizures. Some have trichorrhexis nodosa. One patient reported at 30 years [17] presented at ten years of age with intention tremor. There was no hyperammonemia or encephalopathy. He was described as having mildly impaired mental development, but had attended regular school, could read, write, drive a car, work in a factory, and father a son.

Abnormalities of the EEG are common. Cerebral atrophy may be evident on computed tomography (CT) or magnetic resonance imaging (MRI). Others may have episodes of hyperammonemic encephalopathy and coma thought to be Reye syndrome or encephalitis. Hyperammonemia is often precipitated by infection and such an episode may be fatal.

A unique finding in patients with variant forms of argininosuccinic aciduria is trichorrhexis nodosa (Figures

27.2, 27.3, 27.4, 27.5, 27.6, and 27.7) [1, 2, 18–20]. These patients may appear hairless at a distance, but there is always at least a fuzz of short hair. More often, they have short dry hair, but never need a haircut. The hair is very friable and breaks off easily. There may be a history of hair on the pillow. Under the microscope, the hair sheaths contain tiny nodules (Figure 27.8). Break points may be seen at the nodules.



Figure 27.3 CGG: Her hair was short and brittle.



Figure 27.2 CGG: A Mexican infant with argininosuccinic aciduria. The dermatitis on the abdomen and chest was unrelated.

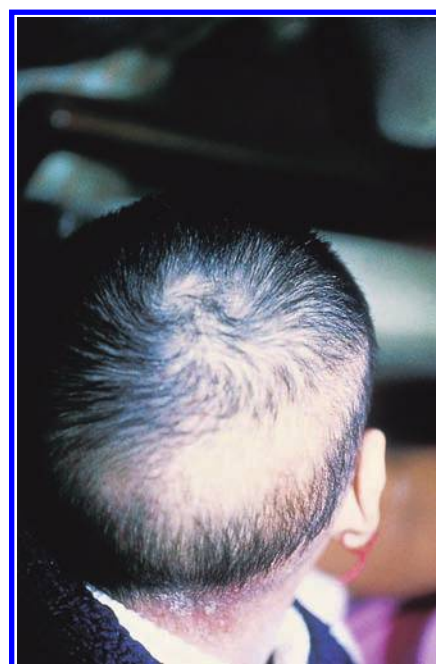


Figure 27.4 CGG: More extensive hair loss at the occipital area of pressure.



Figure 27.5 HH: An infant with argininosuccinic aciduria had brittle hair and alopecia.



Figure 27.7 RW: When the hair was plentiful. (Illustration kindly provided by Dr EV Bawle of the Children's Hospital of Michigan.)



Figure 27.6 RW: A girl with argininosuccinase deficiency [22] who had considerable hair loss during a period of metabolic imbalance. (Illustration kindly provided by Dr EV Bawle of the Children's Hospital of Michigan.)

In addition, these patients have hepatomegaly [21]. Serum values of the transaminases are elevated at least at times of hyperammonemia [21, 22]. Hepatic fibrosis has been diagnosed and there may be ultrastructural abnormalities in hepatocytes. Synthetic functions are usually normal. Chronic coagulopathy was reported in an eight-year-old (Figures 27.6 and 27.7) with prolongation of prothrombin time and increase of the partial thromboplastin time (PTT); the only clinical consequence in the patient reported was prolonged bleeding at venepuncture sites [22].



Figure 27.8 CGG: Trichorexis nodosa.

There may be impairment of physical, as well as mental, development. Patients surviving episodes of coma at any age are likely to be left with impaired mental development. Some have had spasticity ataxia or a seizure disorder.

Hypertension has been observed in patients with argininosuccinic aciduria. This has been thought to relate to nitric oxide synthesis. Citrulline and arginine are the precursor of nitric oxide, which is relevant to smooth muscle arteriolar function.

Argininosuccinic aciduria has been reported in patients, detected by routine neonatal screening, and treated with protein restriction and or/arginine supplementation, in whom no clinical abnormalities have been observed [23–25].

The diagnostic metabolic characteristic is argininosuccinic aciduria. Hyperammonemia may be massive in the neonatal form. In patients with variant forms of the disease, it is usually episodic and less dramatically elevated. Plasma concentrations of glutamine and alanine are usually elevated. Argininosuccinic acid is regularly found in the urine, but this compound is so efficiently excreted that it is not usually found in the blood [26]. High levels are found in the cerebrospinal fluid. In the urine, argininosuccinic acid is excreted in gram quantities. Recently obtained values for arginine succinic acid and its anhydride in the urine ranged from 1163 to 6060 mmol/mol creatinine [17]. However, it may sometimes be missed on routine assays of the urine for amino acids because the compound is unstable, the peaks occur in a place unfamiliar to the operator, or they may overlap those of other amino acids. The best way to assay for argininosuccinic acid is to boil the urine; this quantitatively converts the compound to its anhydrides, which are then readily seen on the amino acid analyzer [27].

GENETICS AND PATHOGENESIS

Argininosuccinic aciduria is transmitted as an autosomal recessive disease. Its incidence approximates 1 in 70,000. The molecular defect is in argininosuccinate lyase (Figure 27.1). This enzyme is widely distributed in tissues and can be assayed in erythrocytes, as well as cultured fibroblasts. Deficient activity of the enzyme has been documented in erythrocytes, liver, and fibroblasts [10, 28–34]. The erythrocyte assay may be misleading; in some patients there may be substantial, even normal, activity even though there is severely defective hepatic activity. The normal enzyme in fibroblasts is immunologically indistinguishable from that of the liver. However, there have been patients reported in whom the activity in fibroblasts was less deficient than that of liver, and others in whom the activity in liver was less deficient than that of fibroblasts [34, 35]. The activity of the enzyme has often been indirectly assayed by determination of the incorporation of ^{14}C -citrulline into proteins. Assay conditions for the enzyme have been improved by the use of a higher concentration of citrulline. In a group of variant patients, this reduced the relationship to normal from 18–75 percent to 6–28 percent [17]. Higher concentrations stimulated incorporation in normal, but not in mutant fibroblasts. The method has also been used for prenatal diagnosis. It also appeared to correlate with the variant phenotypes, in which greater activity was demonstrable.

Heterogeneity in the mutations responsible for deficient enzyme activity in argininosuccinic aciduria was demonstrated in complementation studies of fibroblasts of 28 patients [36], in which there was a single major complementation group, but there were 12 interallelic complementation subgroups consistent with 12 allelic mutations. The enzyme is a homotetramer in which the

monomeric subunit has a molecular weight of 49.5 kDa [37, 38]. Immunochemical studies of the enzyme after electrophoresis on sodium dodecylsulfate polyacrylamide gel electrophoresis revealed two bands of approximately 49 and 51 kDa in normal cells [39]. Each of 28 variants had some 49 kDa cross-reacting material (CRM). The 51-kDa band was found in only six variants in which CRM or residual enzyme activity was very high.

These data were consistent with the existence of a number of unique mutations. Definition of the nature of mutation has supported these conclusions. In four independent cell lines, six mutations were found: three missense mutations, one nonsense mutation, and two deletions [13]. The missense mutations were R111W (arginine 111 to tryptophan), Q286R (glutamine 286 to arginine), and R193Q (arginine 193 to glutamine). In addition, an R95C (arginine 45 cysteine) change was found in a product of consanguinity [14], which when expressed in COS cells exhibited a normal amount of mRNA and only 1 percent of normal enzyme activity. The nonsense mutation changed glycine 454 to X or termination. Two deletions were found that led to skipping of exon 13 [40]. A 13-bp deletion within exon 13 is the most common mutation identified to date, occurring in 8 percent of mutant alleles. The other, a 25-bp deletion, begins at exactly the same spot, which appears to be a hot spot for deletions. The deletions begin with a restriction endonuclease Topo II recognition sequence and start at the Topo II cut site, a site very similar to the DF508 deletion in cystic fibrosis and somewhat similar sites in hypoxanthine phosphoribosyl-transferase (HPRT) and β -globin. In a series of five variant patients [17], three novel mutations (R385C in two patients, V178M, and R379C) were detected in homozygous condition. One patient was a compound of R193Q and Q286R. In 27 unrelated patients, 23 mutations were identified [15], 19 of them novel; 15 of 54 alleles contained the IVS5+1G>A splice site mutation. In 12 Italian patients, 16 different mutations were found, 14 novel [41]. Genotype phenotype correlations remain elusive [17, 41].

Parents of infants with the disease have been found to have reduced activity of argininosuccinate lyase in erythrocytes and fibroblasts [42]. Prenatal diagnosis may be carried out by analysis of the activity of the enzyme in cultured amniocytes [42–44]. Contamination with mycoplasma could cause a false-negative result [14]. Prenatal diagnosis in variant families has been accomplished by ^{14}C -citrulline incorporation in amniocytes and chorionic villus cells [17]. The disease may also be detected prenatally by direct assay of the amniotic fluid for argininosuccinic acid [32, 33, 43–46]. It would seem reasonable always to undertake the direct assay in pregnancies at risk. In a family in which the mutation is known, prenatal diagnosis and carrier detection may be carried out by analysis for the mutation. Neonatal screening was proposed which made use of a *Bacillus subtilis* auxotroph in a Guthrie analysis of blood spots on paper [47], but experience has not been reported. In the Massachusetts program, which

depended on paper chromatography of urine at 3–4 weeks of age, eight patients were found in some 600,000 samples indicating the prevalence of one in 70,000 [48]. Of course, some infants may have died earlier. These approaches to neonatal screening have been replaced by programs of expanded screening by tandem mass spectrometry [49].

The argininosuccinase protein has a structural function first evident from homology with the D-crystallins of avian lens [50, 51]. Duck lens proteins turned out to have enormous argininosuccinate lyase activity. In birds, urea synthesis does not take place; the enzyme is required for the biosynthesis of arginine. In some birds, like chickens, evolutionary divergence has occurred and the major crystallin does not have lyase activity.

TREATMENT

The acute management of the initial hyperammonemic episode and subsequent episodic attacks is set out in [Chapter 29](#), along with principles of the long-term management of hyperammonemic infants. In the acute management of argininosuccinic aciduria, the intravenous sustaining dose of arginine is increased to 700 mg/kg. The priming dose is given in 25 mL glucose solution/kg over 24 hours, and the sustaining dose is given in maintenance fluid over 24 hours. Because arginine is supplied as the hydrochloride for intravenous use, blood levels of chloride and bicarbonate are monitored, and hyperchloremic acidosis is treated with sodium bicarbonate. The use of sodium benzoate and phenylacetate usually may be omitted. The infant with argininosuccinic aciduria can be managed, except at times of crisis, using arginine alone and a diet modestly restricted in protein ([Figure 27.9](#)).

The principle is that if ornithine molecules can be provided through supplemental arginine in order to keep the urea cycle operating, waste nitrogen can be adequately eliminated in the form of argininosuccinic acid. Argininosuccinic acid contains two more N atoms

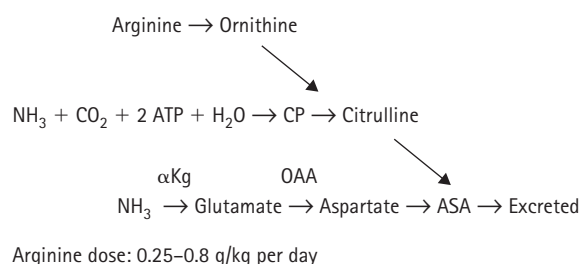


Figure 27.9 Chronic management of argininosuccinic acidemia. Of the four N atoms of argininosuccinic acid, two came from ornithine supplied as arginine. Thus, there is a net loss of two N for every molecule of argininosuccinic acid excreted, which is as efficient as urea. Continued supply of ornithine permits this minicycle to continue to operate. CP, carbamyl phosphate; αKg , α -ketoglutarate; OAA, oxaloacetate; ASA, argininosuccinate.

than the two provided as ornithine, and it is very efficiently excreted, so that it should be as effective as urea in getting rid of unwanted nitrogen, as long as there is a supply of ornithine to keep the cycle moving. As in other urea cycle disorders, an objective of therapy is to keep the levels of glutamine in normal range. Arginine therapy should be sufficient except at times of catabolism, such as during intercurrent infection. If the patient is vomiting or cannot take oral arginine, admission to hospital for parenteral arginine is mandatory. Doses of arginine employed have ranged from 0.25 to 0.89 g/kg per day.

Prognosis for intellectual development probably depends on the nature of the initial hyperammonemia, especially its duration [52] or the nature of recurrent episodes. One should expect patients rescued from neonatal hyperammonemia to have impaired mental development. The mean IQ reported was approximately 50 [52]. The ability to prevent hyperammonemia by early treatment in patients diagnosed prenatally or during neonatal screening should be consistent with a better prognosis.

REFERENCES

- Levin B, Mackay HMM, Oberholzer VG. Argininosuccinic aciduria, an inborn error of amino acid metabolism. *Arch Dis Child* 1961; **36**: 622.
- Carson NAJ, Neill DW. Metabolic abnormalities detected in a survey of mentally backward individuals in Northern Ireland. *Arch Dis Child* 1962; **37**: 505.
- Schreier K, Leuchte G. Argininbernsteinsäure-Krankheit. *Deutsch Med Wschr* 1965; **90**: 864.
- Blackmore RJ, Lyon ICT, Veale AMO. Argininosuccinic aciduria. *Proc U Otago Med Sch* 1972; **50**: 4.
- Carton D, DeShrijver F, Kint J *et al*. Argininosuccinic aciduria. Neonatal variant with rapid fatal course. *Acta Paediat Scand* 1969; **58**: 528.
- Levin B, Dobbs RH. Hereditary metabolic disorders involving urea cycle. *Proc R Soc Med* 1968; **61**: 773.
- Levin B. Hereditary metabolic disorders of the urea cycle. *Adv Clin Chem* 1971; **14**: 65.
- Hambraeus L, Hardell LI, Westphal O *et al*. Argininosuccinic aciduria: report of three cases and the effect of high and reduced protein intake on the clinical state. *Acta Paed Scand* 1974; **63**: 525.
- Farriaux J, Pieraert C, Fontaine G. Survival of infant with argininosuccinic aciduria to 3 months of age. *J Pediatr* 1975; **86**: 639.
- Glick NNR, Snodgrass PJ, Schaer IA. Neonatal argininosuccinic aciduria with normal brain and kidney, but absent liver argininosuccinate lyase activity. *Am J Hum Genet* 1976; **28**: 22.
- O'Brien WE, McInnes R, Kalumuck K, Adcock M. Cloning and sequence analysis of cDNA for human argininosuccinate lyase. *Proc Natl Acad Sci USA* 1986; **83**: 7211.
- Todd S, McGill JR, McCombs JL *et al*. cDNA sequence, interspecies comparison and gene mapping analysis of argininosuccinate lyase. *Genomics* 1989; **4**: 53.

13. Barbosa P, Cialkowski M, O'Brien WE. Analysis of naturally occurring and site-directed mutations in the argininosuccinate lyase gene. *J Biol Chem* 1991; **266**: 5286.
14. Walker DC, McCloskey DA, Simard LR, McInnes RR. Molecular analysis of human argininosuccinate lyase: mutant characterization and alternative splicing of the coding region. *Proc Natl Acad Sci USA* 1990; **87**: 9625.
15. Linnebank M, Tschiedel E, Haberie J *et al*. Argininosuccinate lyase (ASL) deficiency: mutation analysis in 27 patients and a completed structure of the human ASL gene. *Hum Genet* 2002; **111**: 350.
16. Shannon DC, Wichser J, Kazemi H. Hyperventilation and hyperammonemia. *Pediatr Res* 1973; **7**: 423.
17. Kleijer WJ, Garritsen VH, Linnebank M *et al*. Clinical, enzymatic, and molecular genetic characterization of a biochemical variant type of argininosuccinic aciduria: prenatal and postnatal diagnosis in five unrelated families. *J Inherit Metab Dis* 2002; **25**: 399.
18. Solitare GB, Shih VE, Nelligan DJ, Dolan TF Jr. Argininosuccinic aciduria: clinical, biochemical, anatomic and neuropathological observations. *J Ment Def Res* 1969; **13**: 153.
19. Maagøe H. Argininosuccinic aciduria. *Dan Med Bull* 1969; **16**: 308.
20. Farrel G, Rauschkolb EW, Moure J *et al*. Argininosuccinic aciduria. *Tex Med* 1969; **65**: 90.
21. Flick JA, Latham PS, Perman JA, Brusilow SW. Hepatic involvement in argininosuccinase deficiency. *Pediatr Res* 1986; **20**: 239A.
22. Bawle EV, Warrier I. Chronic coagulopathy in a patient with argininosuccinase deficiency. *J Inherit Metab Dis* 1991; **14**: 109.
23. Shih VE. Early dietary management in an infant with argininosuccinase deficiency: preliminary report. *J Pediatr* 1972; **80**: 645.
24. Applegarth DA, Davidson AGF, Perry TL *et al*. Argininosuccinic acidemia in a healthy infant detected by urine screening program. *Clin Chem* 1975; **21**: 950.
25. Shih VE, Coulombe JT, Carney MM *et al*. Argininosuccinic aciduria detected by routine screening. *Pediatr Res* 1976; **10**: 371.
26. Tomlinson S, Westall RG. Argininosuccinic aciduria. Argininosuccinase and arginase in human blood cells. *Clin Sci* 1964; **26**: 261.
27. Nyhan WL, Sakati NO. Argininosuccinic aciduria. In: Nyhan WL, Sakati NO (eds). *Diagnostic Recognition of Genetic Disease*. Philadelphia: Lea and Febiger, 1987: 165.
28. Colombo JP, Baumgartner R. Argininosuccinate cleavage enzyme of the kidney in argininosuccinic aciduria. In: Proceedings of the 6th Annual Symposium, Society for the Study of Inborn Errors of Metabolism, Zurich 1968. London: Churchill-Livingstone, 1969: 19.
29. Batshaw ML, Painter MJ, Sproul GT *et al*. Therapy of urea cycle enzymopathies: three case studies. *Johns Hopkins Med J* 1981; **146**: 34.
30. Shih VE, Littlefield JW, Moser HW. Argininosuccinase deficiency in fibroblasts cultured from patients with argininosuccinase aciduria. *Biochem Genet* 1969; **3**: 181.
31. Hill HZ, Goodman SI. Detection of inborn errors of metabolism. III. Defects in urea cycle metabolism. *Clin Genet* 1974; **6**: 79.
32. Goodman SI, Mace JW, Turner B, Garrett WJ. Antenatal diagnosis of argininosuccinic aciduria. *Clin Genet* 1973; **4**: 236.
33. Jacoby LB, Littlefield JWR, Milunsky A *et al*. A microassay for argininosuccinase in cultured cells. *Am J Hum Genet* 1972; **24**: 321.
34. Pollitt RJ. Argininosuccinate lyase levels in blood, liver and cultured fibroblasts of a patient with argininosuccinic aciduria. *Clin Chim Acta* 1973; **46**: 33.
35. VanderHeiden C, Gerards, LJ VanBiervliet JPGM *et al*. Lethal neonatal argininosuccinate lyase deficiency in four children from same sibship. *Helv Paediat Acta* 1976; **31**: 407.
36. McInnes RR, Shih V, Chilton S. Interallelic complementation in an inborn error of metabolism: genetic heterogeneity in argininosuccinic acid lyase deficiency. *Proc Natl Acad Sci USA* 1984; **81**: 4480.
37. O'Brien WE, Barr BH. Argininosuccinate lyase: purification and characterization from human liver. *Biochemistry* 1981; **20**: 2056.
38. Palekar AG, Mantagos S. Human liver argininosuccinase purification and partial characterization. *J Biol Chem* 1981; **256**: 9192.
39. Simard L, O'Brien WE, McInnes RR. Argininosuccinate lyase deficiency: evidence for heterogeneous structural gene mutations by immunoblotting. *Am J Hum Genet* 1986; **39**: 38.
40. McInnes RR, Christodoulou J, Craig HJ, Walker DC. A deletion 'hotspot' in the argininosuccinate lyase (ASAL) gene has both a TOPO II recognition site and a DNA polymerase α (POL α) mutation site. *Pediatr Res* 1993; **33**: 131A (Abstr. 769).
41. Trevisson E, Salviati L, Baldoin MC *et al*. Argininosuccinate lyase deficiency: mutational spectrum in Italian patients and identification of a novel ASL pseudogene. *Hum Mutat* 2007; **28**: 694.
42. Fleisher LD, Rassin DK, Desnick RH *et al*. Argininosuccinic aciduria: prenatal studies in a family at risk. *Am J Hum Genet* 1979; **31**: 439.
43. Fensom AH, Benson PF, Baker JE, Mutton DE. Prenatal diagnosis of argininosuccinic aciduria: effect of mycoplasma contamination on the indirect assay for argininosuccinate lyase. *Am J Hum Genet* 1980; **32**: 761.
44. Shih VE, Littlefield JW. Argininosuccinase activity in amniotic fluid cells. *Lancet* 1970; **2**: 45.
45. Dhondt JL, Farriaux JP, Pollitt RJ *et al*. Attempt at antenatal diagnosis of argininosuccinic aciduria. *Ann Genet* 1973; **19**: 23.
46. Hartlage PL, Coryell ME, Hall WK, Hahn DA. Argininosuccinic aciduria: prenatal diagnosis and early dietary management. *J Pediatr* 1974; **85**: 86.
47. Talbot HW, Sumlin AB, Naylor EW, Guthrie R. A neonatal screening test for argininosuccinic acid lyase deficiency and other urea cycle disorders. *Pediatrics* 1982; **70**: 526.
48. Levy HL, Coulombe JT, Shih VE. Newborn urine screening. In: Bickel H, Gunthrie R, Hammersen G (eds). *Neonatal Screening for Inborn Errors of Metabolism*. Berlin: Springer-Verlag, 1980: 89.

49. Rashed MS, Rahbeeni Z, Ozand PT. Screening blood spots for argininosuccinase deficiency by electrospray tandem mass spectrometry. *Southeast As J Trop Med Publ Health* 1999; **30**(Suppl. 2): 170.
50. Wistow G, Piatigorsky J. Recruitment of enzymes as lens structural proteins. *Science* 1987; **236**: 154.
51. Lee HJ, Chiou SH, Chang GG. Biochemical characterization and kinetic analysis of duck delta-crystallin with endogenous argininosuccinate lyase activity. *Biochem J* 1992; **283**: 597.
52. Msall M, Batshaw ML, Suss R *et al.* Neurologic outcome in children with inborn errors of urea synthesis. *N Engl J Med* 1984; **301**: 1500.

Argininemia

Introduction	223	Treatment	226
Clinical abnormalities	223	References	227
Genetics and pathogenesis	225		

MAJOR PHENOTYPIC EXPRESSION

Spastic quadriplegia, opisthotonus, convulsions, microcephaly, psychomotor impairment, hyperargininemia, argininuria and secondary cystinuria, lysinuria and ornithinuria, orotic aciduria, and deficiency of arginase.

INTRODUCTION

Argininemia is a disorder in which the clinical picture is quite different from the other disorders of the urea cycle. The picture is that of a spastic diplegia or quadriplegia [1–3]. It was reported in 1965 by Serrano [1] and in 1969 by Terheggen and colleagues [2]. The disease is caused by a virtually complete absence [4, 5] of the activity of arginase (EC 3.5.3.1) (Figure 28.1). The human and rat genes have been cloned [6, 7]. The human gene *ARG1* is located on chromosome 6 at band q23 [7]. A small number of mutations have been heterogeneous [8, 9].

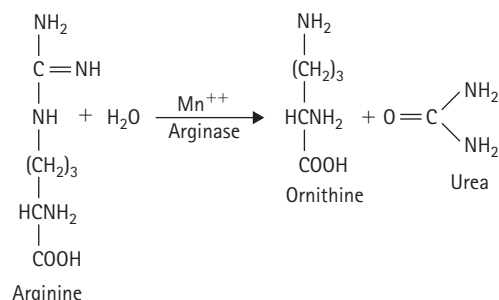


Figure 28.1 The reaction catalyzed by arginase.

CLINICAL ABNORMALITIES

Patients with argininemia are often recognized as abnormal because of failure to pass developmental milestones. With

the advent of spasticity or opisthotonus, they may be first thought to have cerebral palsy [3, 10–20]. Onset may be with convulsions in the neonatal period [1–3]. Some patients may have recurrent cyclic vomiting from the early days of life [21]. Others may display anorexia, irritability, or inconsolable crying; some patients have failure to thrive. Alternatively, there may be no signs in early infancy until it is apparent that development is delayed. The mother of one infant remarked on her drowsiness after feeding [3]. Protein intolerance has been observed very early in life [19].

In the established phenotype, the patient has marked spasticity and frequently opisthotonic (Figures 28.2 and 28.3). If walking is possible, the gait is a spastic toe-walk. Scissoring of the lower extremities is common. Muscle tone is hypertonic, and the deep tendon reflexes are accentuated, both usually more so in the legs than in the arms. Patients may be hyperactive or irritable. They may be ataxic or appear clumsy. Involuntary movements may be choreic or athetoid, or there may be tremors. Drooling and dysphagia are common. Convulsions are regularly observed and abnormalities of the electroencephalogram (EEG) are the rule [22]. The pattern of the EEG may be that of a spike and wave. Patients often develop microcephaly (Figure 28.4) and cerebral atrophy is visible on computed tomography (CT) or magnetic resonance imaging (MRI). Psychomotor impairment is usually severe, but it may be minimal in patients diagnosed and treated early (Figure 28.2).

Patients with argininemia may have episodic vomiting and hyperammonemia. Some have symptomatic hyperammonemia progressive to coma, and death in infancy



Figure 28.2 TG: A 19-year-old girl with argininemia. She walked with a distinct spastic gait and had equinovarus posturing of the feet. Deep tendon reflexes were brisk and there was clonus at both ankles.

has been reported [19, 23]. In one patient, hypertonicity, tachypnea, lip smacking, and right-sided bicycling movements at 30 hours heralded fatal cerebral edema [23]. Also following an acute hyperammonemic episode, neurologic function may further deteriorate. Abnormal concentrations of ammonia are less commonly encountered than in other disorders of the enzymes of the urea cycle. However, in a report [24] of an infant who presented at five months with an ammonia concentration of 736 $\mu\text{mol/L}$ and cerebral edema, experience was reported with eight other patients with recurrent ammonia elevation.

Concentrations of ammonia may be elevated only intermittently and hyperammonemia when it occurs, tend to be moderate. In some patients, the levels of ammonia were elevated in both the fed and fasting states [2]. In an occasional patient, the concentration of ammonia may be surprisingly high in the absence of obvious symptoms of hyperammonemia [3]. On the other hand, in the patient with neonatal cerebral edema, the peak ammonia was only 114 mmol/L , and it fell to 18 mmol/L with only supportive therapy [23]. There may be hepatomegaly.

One patient was first diagnosed at 18 years of age when

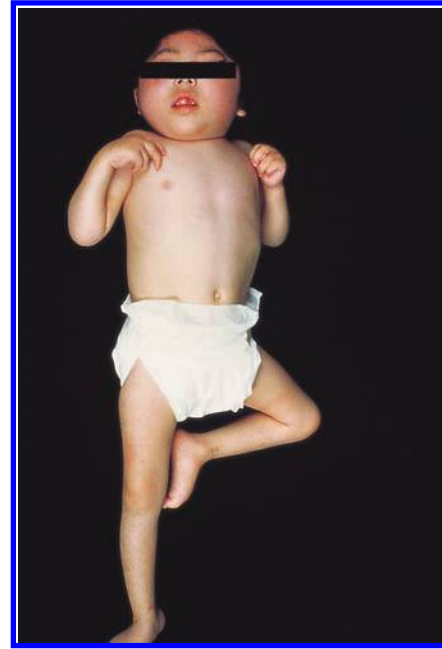


Figure 28.3 A patient with argininemia has spasticity, often opisthotonic, had severely impaired mental development and microcephaly. Convulsions began at 23 days of age. By four years, she had no head control. (This illustration was kindly provided by Dr Makato Yoshino of Kurume University School of Medicine, Kurume, Japan.)



Figure 28.4 The microcephaly of the patient. Neuroimaging revealed cerebral atrophy. Concentrations of arginine ranged from 3.1 to 19.4 mg/dL . Orotic aciduria was 6900 mg/mg creatinine. (This illustration was kindly provided by Dr Makato Yoshino of Kurume University School of Medicine, Kurume, Japan.)

he developed hyperammonia after initiation of treatment for seizures with valproic acid [25]. Hyperammonia after valproic acid has also been observed in ornithine transcarbamylase deficiency (Chapter 24) and citrullinemia (Chapter 26).

Serum activities of the transaminases may be elevated and the prothrombin time may be prolonged. Unusual zones of different coloration and fragility of the hair have been reported in one patient [1]. Biopsied liver has been reported [4] to reveal multifocal hydropic changes.

Patients are generally recognized by the assessment of the concentration of amino acids in the blood or urine, the latter of which may be mistaken to be that of cystinuria (Chapter 70). All such abnormal findings should be pursued by the quantitative analysis of amino acids of the plasma. In patients with argininemia, the plasma concentration of arginine is usually four to 20 times that of the normal individuals, often up to 1500 $\mu\text{mol/L}$ [14]. However, in one patient with very severe deficiency and lethal clinical disease the value was only 170 $\mu\text{mol/L}$ [19].

Concentrations of arginine in cerebrospinal fluid (CSF) are also markedly elevated [3]. The concentration of the glutamine may be also increased, especially in the acute hyperammonemic crisis. In the neonate with cerebral edema [23], the plasma glutamine was 909 mmol/L and that of the CSF was 9587 mmol/L. Concentrations of other amino acids may also be elevated in the CSF [4, 20]. These include ornithine, aspartate, threonine, glycine, and methionine. A mechanism for their increase is not clear. The excretion of arginine in the urine is substantial. The urine also contains increased quantities of lysine, cystine, and ornithine; this is the result of competition for their renal tubular reabsorption by the large amounts of arginine being processed by the kidney [22]. The amounts of cystine and ornithine are usually relatively less than observed in cystinuria. Lowering of plasma concentrations of arginine, by restriction of intake of protein, effectively reverses this pattern of urinary amino acid excretion.

GENETICS AND PATHOGENESIS

Argininemia is an autosomal recessive disease [16]. It has been reported about equally in males and females, and parents are unaffected. Incidence is approximately one in 36,300. The molecular defect is in the enzyme arginase. It catalyzes the conversion of arginine to urea and ornithine (Figure 28.1). The activity of the enzyme is readily measured in erythrocytes and it is through assay in this tissue that the diagnosis is usually made [3, 4, 10]. The defect has also been demonstrated in liver [5]. The enzyme is not expressed in cultured fibroblasts [26]. The enzyme in red cells appears to be identical to that in liver. The enzyme from rat liver has been crystallized. It is a trimer of three 35-kDa monomers [27]. Negligible amounts of mRNA are normally found in tissues other than liver and erythrocytes.

Immunochemical studies using antibody to normal

human hepatic arginase have shown cross-reacting material (CRM) in liver and erythrocytes of patients with argininemia in amounts equivalent to those normal individuals, indicating that a catalytically inactive structural arginase protein is made [28]. On the other hand, Western blot analyses of 15 patients were reported to reveal detectable arginase protein in only two patients [18]. Biopsy of the kidney of two patients with argininemia revealed arginase activity that was considerably greater than that of controls. Kidney contains a distinct mitochondrial arginase whose gene does not hybridize with that of the hepatic gene that is affected in argininemia [29]. The activity of the renal arginase provides the mechanism for the relatively normal production of urea in these patients. The renal enzyme is 58 percent identical to the hepatic enzyme and 70 percent identical to *Xenopus* arginase.

The human gene at chromosome 6q23 is 11.5 kb in size and contains 18 exons [30]. The crystal structure of the enzyme has been elucidated [31]. A dimagnesium cluster is essential for enzymatic activity and stability of the protein [32]. Mutation analysis revealed no gross deletions by Southern blot analysis in 15 patients [8]. In three, a *TaqI* restriction enzyme cleavage site was missing. In two of these, mutations were identified. One was homozygous for R291Y (an arginine-to-tyrosine change) and the other heterozygous for T290S (a threonine-to-serine change). A Japanese patient was found to be a compound in which on one allele there was a four-base deletion in exon 3, which caused a frame shift at position 87 and a premature termination 45 residues later, while on the other allele a single base deletion in exon 2 led to a frame shift at 26 and premature termination on five residues later [9]. In another study of Japanese patients [33], the mutations found were in W122X, 6235R, and L282 frame shift. The enzyme activities assayed in expression studies in *E. coli* were zero, consistent with enzyme assays in the erythrocytes of patients. Additional mutations identified include D128G and H141L [34]. Mutations in AARG1 though heterogeneous have largely been point mutations rather than major or structural alternations in the gene [8].

Detection of heterozygotes has been accomplished by finding arginase levels in erythrocytes or leukocytes that were appreciably less than the control levels [12, 16, 24]. The leukocyte concentration of arginine has been used to distinguish heterozygotes when arginase levels were not useful. Mutation analysis is preferred for this purpose when the mutation is known. Prenatal diagnosis has been difficult because the enzyme is not expressed in fibroblasts [24]. Direct measurement by gas chromatography-mass spectrometry (GCMS) of orotic acid in amniotic fluid [35] was not useful in ornithine transcarbamylase deficiency, but it could be more reliable in argininemia. Fetal blood sampling can be employed, but with risk of fetal loss. If the mutation is known, this is the method of choice in prenatal diagnosis. Linkage analysis is available which takes advantage of restriction length polymorphism (RFLP) to *PvuII* and should be useful in prenatal diagnosis [36] and

so should a dinucleotide repeat polymorphism [34, 37].

A screening method was developed that permits routine screening of newborns for deficiency of arginase [38, 39]. The advent of tandem mass spectrometry and its applications to newborn screening has supplanted this approach.

In the presence of defective activity of arginase, there is in addition to the accumulation of arginine, an impressive orotic aciduria [4, 40]. The amounts of orotic acid found in the urine are considerably greater than those of patients with argininosuccinic aciduria and occur in the absence of hyperammonemia. So, this is not a consequence of accumulation of carbamylphosphate behind the block, as occurs in ornithine transcarbamylase deficiency. Rather, it appears to be the direct result of the stimulation by accumulated arginine of N-acetylglutamate synthetase (NAGS), which leads to increased synthesis of carbamylphosphate (Figure 28.5) [40]. Arginine is a normal activator of NAGS. This accumulation of carbamylphosphate when arginase is deficient leads preferentially to the biosynthesis of pyrimidines. Consistent with this were the low levels of ornithine reported by Yoshino *et al.* [3], and the fact that, as ornithine levels were increased by treatment, the excretion of orotic acid decreased to normal levels, even though the concentration of arginine rose considerably. The orotic aciduria in this condition is also associated with increased excretion of uridine and uracil [41].

N-Acetylarginine, α -keto-guanidinovaleric acid, and argininic acid, direct derivatives of arginine, are also found in the urine in this disorder, as well as guanidinoacetic acid and guanidinobutyric acid, compounds in which the amino group is donated via transamidation reaction [12, 13, 42, 43]. Guanidinosuccinic acid excretion is not increased, whereas it does increase in individuals given an arginine load, suggesting a role for arginase in the generation of this compound [44, 45]. The serum concentration of urea is usually normal in these patients.

The pathogenesis of the neurologic disability in argininemia is not clear, but doubtless it is the result of the chemical milieu in which the patient's brain develops. Intermittent or chronic elevation of ammonia could be sufficient, but the phenotype is so different from that of the

other defects of the urea cycle that something about arginine or its products must have effects on the central nervous system. Neurotransmitter metabolism has been reported to be impaired in argininemia [46]. The production of nitric oxide from arginine could be another factor.

The occurrence of high levels of glutamine, especially in the CSF in the neonate with cerebral edema and only modest hyperammonemia [23] is consistent with a role for glutamine in this complication.

An arginase-deficient mouse displayed growth deficiency and hyperammonemia that led to death by 12 days of life [47].

TREATMENT

Nutritional therapy has been designed to keep levels of arginine within normal limits, and success has been reported not only in meeting this objective, but also in promoting normal neurologic development [4, 16, 17, 48–53]. The methods employed have included protein restriction [13] and the use of mixtures of amino acids excluding arginine [16, 50]. The latter approach has been effective in controlling levels of arginine in a patient treated from birth [51], as well as in older individuals [16]. Supplementation with lysine raised low levels of lysine in serum, but concentrations of arginine in plasma and CSF increased and concentrations of ornithine in the CSF fell [50]. Supplementation with ornithine improved levels of ornithine and had a pronounced effect in lowering the amounts of orotic acid in the urine [3, 50]. During combined supplementation with lysine and ornithine, a patient gained weight well and epileptiform activity on the EEG improved [50]. In one report [48], nitrogen-free analogs of some essential amino acids were employed to minimize further the nitrogenous sources of arginine in a low arginine diet.

Sodium benzoate therapy was employed in a 15-year-old patient with progressive spastic diplegia and borderline intelligence who had numerous hyperammonemic episodes, and required nasogastric tube feeding to maintain nutrition [52]. The doses employed were 250–375 mg/kg. This approach controlled levels of ammonia and reduced

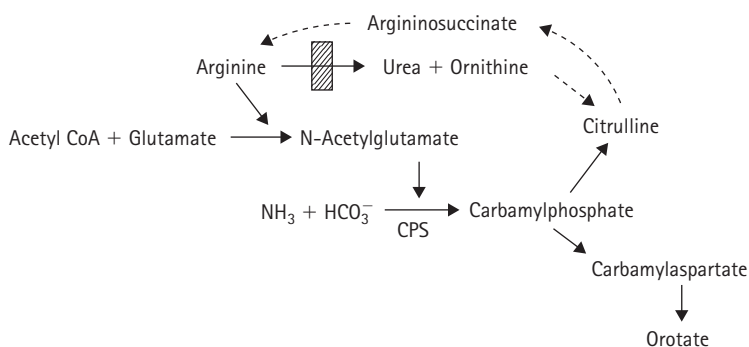


Figure 28.5 Pathogenesis of the orotic aciduria of argininemia. Accumulation of arginine provides an effector function on N-acetylglutamate synthetase, and the product of this reaction stimulates carbamylphosphate synthetase (CPS). The carbamylphosphate generated does not accumulate, and since in the absence of arginase, ornithine is limiting, it flows along the pathway of pyrimidine synthesis to orotic acid.

plasma concentrations of arginine. Restriction of the dietary intake of arginine reduced levels further. The excretion of orotic acid decreased to normal levels. Urinary hippurate excreted amounted to 60–80 percent of the administered benzoate and this constituted 35–43 percent of the urinary nitrogen. Progression of the diplegia was thought to have stopped. Sodium benzoate was also efficacious in a 12-year-old patient with less severe disease who had self-selected a diet low in protein [53]. Phenylbutyrate or phenylacetate should have similar effects, but the resulting odor is less acceptable socially.

Phenylbutyrate increases expression of some genes and it has been reported [54] to increase the activity of arginase in mice and in cultured cells. It was not tested in cells of patients with argininemia, but might be useful in patients with residual activity.

REFERENCES

- Serrano AP. Argininuria, convulsiones y oligofrenia: un nuevo error innato del metabolismo? *Rev Clin Esp* 1965; **97**: 176.
- Terheggen HG, Schwenk A, Lowenthal A *et al*. Argininaemia with arginase deficiency. *Lancet* 1969; **2**: 748.
- Yoshino M, Kobota K, Yoshida I *et al*. Argininemia: report of new mechanisms of orotic aciduria and hyperammonemia. In: Lowenthal A, Mori A, Marescau B (eds). *Urea Cycle Diseases*. Advances in Experimental Medicine and Biology, vol. 153. New York: Plenum Press, 1982: 121.
- Cederbaum SD, Shaw KNF, Spector EB *et al*. Hyperargininemia with arginase deficiency. *Pediatr Res* 1979; **13**: 827.
- Michels VV, Beaudet AL. Arginase deficiency in multiple tissues in argininemia. *Clin Genet* 1978; **13**: 61.
- Dizikes GJ, Grody WW, Kern RM, Cederbaum SD. Isolation of human arginase cDNA and absence of homology between two arginase genes. *Biochem Biophys Res Commun* 1986; **141**: 53.
- Sparkes RS, Dizikes GJ, Klisak I *et al*. The gene for human liver arginase (ARGI) is assigned to chromosome band 6q23. *Am J Hum Genet* 1986; **39**: 186.
- Grody WW, Klein D, Dodson AE *et al*. Molecular genetic study of human arginase deficiency. *Am J Hum Genet* 1992; **50**: 1281.
- Haraguchi Y, Aparicio JM, Takiguchi M *et al*. Molecular basis of argininemia. Identification of two discrete frame-shift deletions in the liver-type arginase gene. *J Clin Invest* 1990; **86**: 347.
- Terheggen HG, Schwenk A, Lowenthal A *et al*. Hyperargininämie mit Arginasedefekt eine neue familiäre Stoffwechselstörung. *I Klin Bef Z Kinderheilk* 1970; **107**: 298.
- Iyer R, Jenkinson CP, Vockley JC *et al*. The human arginases and arginase deficiency. *J Inherit Metab Dis* 1998; **21**: 86.
- Terheggen HG, Lavinha F, Colombo JP *et al*. Familial hyperargininemia. *J Hum Genet* 1972; **20**: 69.
- Terheggen HG, Lowenthal A, Lavinha F, Colombo JP. Familial hyperargininaemia. *Arch Dis Child* 1975; **50**: 57.
- Terheggen HG, Schwenk A, Lowenthal A *et al*. Hyperargininämie mit Arginasedefekt eine neue familiäre Stoffwechselstörung. II Biochemische Untersuchungen. *Z Kinderheilk* 1970; **107**: 313.
- Van Sande M, Terheggen HG, Clara R *et al*. Lysine-cystine pattern associated with neurological disorders. In: Carson NAJ, Raine NAJ (eds). *Inherited Disorders of Sulfur Metabolism*. Edinburgh: Churchill Livingstone, 1971: 85.
- Snyderman SE, Sansaricq CC, Cheu WJ *et al*. Argininemia. *J Pediatr* 1977; **90**: 563.
- Cederbaum SD, Shaw KNF, Valente M. Hyperargininemia. *J Pediatr* 1977; **90**: 569.
- Qureshi IA, Letarte J, Ouellet R *et al*. Ammonia metabolism in a family affected by hyperargininemia. *Dia Metab* 1981; **7**: 5.
- Jorda A, Rubio V, Portoles M *et al*. A new case of arginase deficiency in a Spanish male. *J Inherit Metab Dis* 1986; **9**: 393.
- Bernar J, Hanson RA, Kern R *et al*. Arginase deficiency in a 12-year-old boy with mild impairment of intellectual function. *J Pediatr* 1986; **108**: 432.
- Nyhan WL, Sakati SA. Argininemia. In: Nyhan WL, Sakati SA (eds). *Diagnostic Recognition of Genetic Disease*. Philadelphia: Lea and Febiger, 1987: 169.
- Terheggen HG, Lowenthal A, Colombo JP. Clinical and biochemical findings in argininemia. In: Lowenthal A, Mori A, Marescau B (eds). *Urea Cycle Diseases*. Advances in Medicine and Biology, vol. 153. New York: Plenum Press, 1982: 111.
- Pickler JD, Puga AC, Levy HL *et al*. Arginase deficiency with lethal neonatal expression: evidence for the glutamine hypothesis of cerebral edema. *J Pediatr* 2003; **142**: 349.
- Ghai S, Nagamani SC, Lee B *et al*. Severe infantile presentation of arginase I deficiency. *Mol Genet Metab* 2011; **102**: 291.
- Christmann D, Hirsch E, Mutschier V *et al*. Argininemie congenitale diagnostiquee tardivement a l'occasion de la prescription de valproate de sodium. *Rev Neurol* 1990; **146**: 764.
- Van Elsen A, Leroy JG. Human hyperargininemia: a mutation not expressed in skin fibroblasts. *Am J Hum Genet* 1977; **29**: 350.
- Kanyo ZF, Chen CY, Daghighi DF *et al*. Crystallization and oligomeric structure of rat liver arginase. *J Mol Biol* 1992; **224**: 1175.
- Spector EB, Rice SCH, Cederbaum SD. Immunologic studies of arginase in tissues of normal human adult and arginase-deficient patients. *Pediatr Res* 1983; **17**: 941.
- Grody WW, Argyle C, Kern RM *et al*. Differential expression of two human arginase genes in hyperargininemia. Enzymatic, pathologic and molecular analysis. *J Clin Invest* 1989; **83**: 602.
- Haraguchi Y, Aparicio JMR, Takiguchi M *et al*. Molecular basis of argininemia: identification of two discrete frame-shift deletions in the liver-type arginase gene. *J Clin Invest* 1990; **86**: 347.
- Kanyo ZF, Scolnick LR, Ash DE, Christianson DW. Structure of a unique binuclear manganese cluster in arginase. *Nature* 1996; **383**: 554.
- Scolnick LR, Kanyo ZF, Cavalli RC *et al*. Altering the binuclear manganese cluster of arginase diminishes thermostability and catalytic function. *Biochemistry* 1994; **36**: 10652.
- Uchino T, Haraguchi Y, Aparicia JM *et al*. Three novel mutations in the liver-type arginase gene in three unrelated

- Japanese patients with Argininemia. *Am J Hum Genet* 1992; **51**: 1406.
34. Vockley JG, Tabor DE, Kern RM *et al.* Identification of mutations (D128G, H141L) in the liver arginase gene of patients with hyperargininemia. *Hum Mutat* 1994; **4**: 150.
35. Jakobs C, Sweetman L, Nyhan WL *et al.* Stable isotope dilution analysis of orotic acid and uracil in amniotic fluid. *Clin Chim Acta* 1984; **143**: 123.
36. Kidd JR, Dizikes GJ, Grody WW *et al.* A Pvu11 RFLP for the human liver arginase (ARG1) gene. *Nucleic Acids Res* 1984; **14**: 9544.
37. Meloni R, Fougerousse F, Roudaut C, Beckmann JS. Dinucleotide repeat polymorphism at the human liver arginase gene (ARG 1). *Nucleic Acids Res* 1992; **20**: 1166.
38. Orfanos AP, Naylor EW, Guthrie R. Fluorometric micromethod for determination of arginase activity in dried blood spots on filter paper. *Clin Chem* 1980; **26**: 1198.
39. Naylor EW, Orfanos AP, Guthrie R. A simple screening test for arginase deficiency (hyperargininemia). *J Lab Clin Med* 1977; **89**: 876.
40. Bachmann C, Colombo JP. Diagnostic value of orotic acid excretion in heritable disorders of the urea cycle and in hyperammonemia due to organic acidurias. *Eur J Pediatr* 1980; **134**: 109.
41. Naylor EW, Cederbaum SD. Urinary pyrimidine excretion in arginase deficiency. *J Inherit Metab Dis* 1981; **4**: 207.
42. Marescau B, Pintens J, Lowenthal A, Terheggen HG. Excretion of alpha-keto-gamma-guanidinovaleic acid and its cyclic form in patients with hyperargininemia patients. *J Hum Genet* 1976; **24**: 61.
43. Wiechert P, Mortelman J, Lavinha F *et al.* Excretion of guanidine-derivatives in urine of hyperargininemic patients. *J Hum Genet* 1976; **24**: 62.
44. Mori A, Matsumoto M, Hiramatsu C. Alpha-guanidinoglutaric acid in urine of arginine loaded rabbits. *IRCS Med Sci Biochem* 1980; **8**: 75.
45. Stein IM, Cohen BD, Kornhauser RS. Guanidino-succinic acid in renal failure experimental azotemia and inborn errors of the urea cycle. *N Engl J Med* 1969; **280**: 926.
46. Hyland K, Smith I, Clayton PT, Leonard JV. Impaired neurotransmitter amine metabolic deficiency. *J Neurosurg Psychiatry* 1985; **48**: 1189.
47. Iyer RK, Yu H, Kern RM *et al.* Further studies on the arginase-1 deficient mouse. *Am J Hum Genet* 2002; **71**: 413.
48. Cederbaum SD, Shaw KNF, Valente M, Cotton ME. Argininosuccinic aciduria. *Am J Mental Def* 1973; **77**: 395.
49. Cederbaum SD, Moedijono SJ, Shaw KNF *et al.* Treatment of hyperargininemia due to arginase deficiency with a chemically defined diet. *J Inherit Metab Dis* 1982; **5**: 95.
50. Kang SS, Wong PWK, Melyn MA. Hyperargininemia: effect of ornithine and lysine supplementation. *J Pediatr* 1983; **103**: 763.
51. Synderman SW, Sansaricq C, Norton PM, Goldstein F. Argininemia treated from birth. *J Pediatr* 1979; **94**: 61.
52. Qureshi IA, Letarte J, Quellet R. Treatment of hyperargininemia with sodium benzoate and arginine-restricted diet. *J Pediatr* 1984; **104**: 473.
53. Bernar J, Hanson RA, Kern R *et al.* Arginase deficiency in a 12-year-old boy with mild impairment of intellectual function. *J Pediatr* 1986; **108**: 432.
54. Kern RM, Yang Z, Grody WW *et al.* Arginase induction by sodium phenylbutyrate in mouse tissues and human cell lines. *Am J Hum Genet* 2002; **71**: 425.

CLINICAL ABNORMALITIES

Intermittent episodes of hyperammonemia are characteristic features of this disorder. This may be manifest in episodic vomiting, lethargy, coma, or ataxia. It was evident in the experience with the initial and subsequent patients that these symptoms vary directly with the dietary intake of protein and the degree of hyperammonemia. This disease may first present as a Reye-like syndrome. Our patient was diagnosed as an example of Reye syndrome prior to referral, despite the fact that coma developed following feeding of 8 g protein/kg following a thermal burn (Figures 29.2 and 29.3).

In infancy, failure to thrive and developmental delay have been observed, although the initial patient grew along the 10th percentile [1], except when fed his lowest intake of protein, and others have grown normally while receiving diets moderately restricted in protein [6]. In our patient, failure to thrive was associated with very low levels of lysine in plasma [7]. Prior to supplementation with lysine orotate, growth in length had virtually ceased. Growth was rewarding following supplementation, which returned concentrations of lysine in plasma to normal.

Ultimate intelligence has ranged from low normal to severely mentally impaired [1–4, 10–13]. In two families, IQ levels ranged from 76 to 80. In one, diagnosis was made as part of an evaluation for poor school performance in otherwise asymptomatic brothers. In the other family, the diagnosis was made at three years of age on the basis of



Figure 29.2 A five-year-old Vietnamese boy with the hyperornithinemia, hyperammonemia, homocitrullinuria (HHH) syndrome. Scars on his legs signify the thermal burns and its attendant treatment with large amounts of protein that led to his only episode of coma and an initial diagnosis of Reye syndrome.



Figure 29.3 Close up reveals no unusual physical features. He was developmentally delayed.

mild hyperammonemia following gastroenteritis, while the 13-year-old sister was asymptomatic; IQ was 79 in both siblings who were doing well in mainstream classes.

Hyperammonemic attacks may be less frequent in older patients, who may select a diet low in protein. On the other hand, a 21-year-old, with severely impaired mental development continued to have stuporous episodes, at least one a month, which lasted up to 2 hours. Seizures have been observed with onset from ten months to 18 years. They may be generalized, tonic-clonic, as well as myoclonic [6, 14]. One patient presented with attacks of headache progressive to unconsciousness beginning at 39 years of age [8]. Our patient has been left with a chronic seizure disorder, despite an absence of symptomatic hyperammonemia since the initial episode of coma. Severe neonatal hyperammonemia is rare in this disease [4]. Cerebella ataxia has also been observed [14, 15].

Ocular findings, in contrast to gyrate atrophy of the retina, have been normal, except for a patient who developed papilledema during an attack of acute symptomatic hyperammonemia [3]. Another patient had retinal depigmentation and choroidal thinning [16], but visual function was normal.

Progressive spastic paraplegia was emphasized as a clinical characteristic in three patients in one family [11]. This was clearly evident in the oldest patient, who began to have progressive disturbance of gait at 14 years, and at 21 had increased deep tendon reflexes, sustained ankle clonus, and bilateral Babinski responses. His IQ was 67. He stuttered and had an aggressive personality that led to psychiatric consultation. His 18-year-old sister had an IQ of 60 and could not run or jump; deep tendon reflexes

were increased and there were ankle clonus and Babinski responses. The 13-year-old brother had brisk deep tendon reflexes and an IQ of 51. Others have been reported with spastic paraplegia [17]. Pyramidal tract signs may be prominent [16].

Cortical and cerebellar atrophy has been described on computed tomography (CT) and magnetic resonance imaging (MRI) [8, 16, 17] and there have been abnormal white matter changes. Liver biopsy of a patient with hepatomegaly [3] revealed abnormal mitochondria containing crystalloid structures. In another patient [12], there was diffuse microvesicular fat and there were large lipid-containing vacuoles, as well as large, irregular mitochondria with paracrystalline inclusions. Our patient [7] had hepatic microvesicular fat, which had appeared to confirm the diagnosis of Reye syndrome. Some patients have had coagulopathy (and liver dysfunction and increased transaminase enzymes) [18, 19].

Metabolic abnormality is usually first evident in hyperammonemia. The levels encountered in an acute attack, even in a patient in coma, are usually considerably less elevated than those we are accustomed to in neonatal infants with disorders of the urea cycle. The concentration of ammonia may be chronically elevated in a patient ingesting a diet high in protein. More often, the level is normal in fasting, but elevated postprandially. The concentrations of glutamine and alanine in plasma may be increased as concomitants of hyperammonemia. Orotic acid excretion has been reported to be elevated in only about half of the patients [3], but it may be induced by loading with protein or alanine. Our patient (Figures 29.2 and 29.3) had little, and often no, measurable urinary orotic acid at baseline, but loading with alanine led to a pronounced

orotic aciduria (Table 29.1). He was a refugee from Vietnam accustomed to a low protein diet. As he gradually became Americanized, his protein intake increased and the amounts of orotic acid in the urine increased progressively (Table 29.2). The initial diagnosis may be made difficult by the increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) that may occur acutely with the hyperammonemia [1, 10].

A woman with this disorder treated successfully during pregnancy with arginine to control ammonia delivered a normal baby whose IQ at five years was 130 [20].

GENETICS AND PATHOGENESIS

Hyperornithinemia is a hallmark feature of the metabolic abnormality of this disease which has been found in over 60 patients. Concentrations in plasma have usually ranged from 270 to 780 $\mu\text{mol/L}$ [1–4, 7]. Concentrations as high as 915 and 1439 $\mu\text{mol/L}$ have been recorded [1, 11]. Ornithinuria has ranged from 73 to 8160 $\mu\text{mol/g creatinine}$. The highest levels of ornithine in body fluids have been those encountered during acute episodes of hyperammonemia. Confronted with an elevated concentration of ornithine and hyperammonemia, especially in a patient with orotic aciduria, one thinks about ornithine transcarbamylase deficiency, but ornithine concentrations are never elevated in ornithine transcarbamylase deficiency, even in those with unusual kinetic properties (Chapter 25) [21]. Oral loading with ornithine in HHH (hyperornithinemia, hyperammonemia, homocitrullinuria) syndrome leads to higher peak levels than in normal individuals and a slower return to baseline [1, 11].

There are two other types of hyperornithinemia: one with gyrate atrophy of the choroid and retina, in which the activity of ornithine-5-aminotransferase is deficient [22], and a disorder reported [23] in two siblings with impaired mental development and renal tubular dysfunction, which may represent a partial deficiency of the same enzyme, because its activity in liver was reported to be 60–80 percent reduced, but kinetically normal [24]. In any case, neither of these hyperornithinemic situations is ever hyperammonemic.

Homocitrullinuria is the third major feature of the disease. In the presence of accumulated carbamylphosphate, lysine is carboxylated to form homocitrulline (Figure 29.1), which is efficiently excreted in the urine. Reported levels of excretion have ranged from 93 to 2380 $\mu\text{mol/g creatinine}$. As in the case of the orotic aciduria, homocitrullinuria may be absent or not prominent in patients receiving little protein in their diets. Its levels of excretion can be correlated with protein intake [13] or the administration of lysine, and good correlation was observed between the urinary homocitrulline:creatinine ratio and the plasma lysine:ornithine ratio [13]. Homocitrulline is commonly found in the urine of infants and children, a consequence of its formation by the heat treatment of milk products, and its

Table 29.1 Excretion of orotic acid following alanine^a

Hours	Mg/g creatine
0–2.5	18
2.5–5	236
5–6	229
6–24	242

^aThe dose of alanine was 400 mg/kg.

Table 29.2 Relationship between the excretion of orotic acid and the intake of protein

Protein intake (g/kg/day)	Urinary orotic acid (mg/g creatine)
2	–
3.7	12
4.7	19
5.4	272

subsequent ingestion and excretion [25,26]. It is often found in patients with generalized aminoaciduria and regularly follows lysine loading in normal children and adults [27,28].

Concentrations of lysine in the blood may be elevated during the acute attack of hyperammonemia as a nonspecific concomitant of hyperammonemia. During steady-state conditions, levels of lysine in blood and urine are usually low [11]. We have observed that lysine may become limiting for growth in this disease [7].

Other unusual compounds may be found in the urine of the patient. Among those identified is 3-aminopiperid-2-one, a cyclic D-lactam or methylester of ornithine [29,30]. Amounts as high as 459 $\mu\text{mol/g}$ creatine have been reported [11].

The molecular defect is in the transport system responsible for the movement of ornithine into mitochondria (Figure 29.1) [5,13]. This makes ornithine limiting for the synthesis of citrulline and impairs the operation of the urea cycle. This transporter was reported by Gamble and Lehninger [31] to be unidirectional and highly stereospecific for L-ornithine. It requires respiratory energy. The driving force for entry of ornithine is a negative internal transmembrane potential produced by the entry of proton-conducting anions. The system was characterized in rat liver mitochondria; it was not operative in heart. Citrulline passes through the membrane in both directions without requiring respiratory energy.

Evidence for a defect in the ornithine transport system was first obtained [6] by the study of ^{14}C -ornithine incubation in intact fibroblasts. Similar results were obtained in studies of fibroblasts incubated with ^{14}C -ornithine and assessment of its incorporation into protein [32]. The apparent K_m for this process in a patient's fibroblasts was ten times that of controls. Direct measurement of amino acid concentrations in hepatic mitochondria of a patient revealed the concentration of ornithine to be low [8]. Direct evidence of defective transport of ornithine was obtained in studies of mitochondria isolated from fresh liver tissue of three patients [5]. Fibroblasts of patients with this disorder were effectively complemented by those of a patient with gyrate atrophy, while the cells of two patients with the HHH syndrome fell into the same complementation group [32]. Inheritance is autosomal recessive.

The ornithine transporter gene *SIC25A15*, which was called *ORNT1*, was identified by the use of sequences from the ARG 11 and ARG 13 genes of *Neurospora* and *Saccharomyces* which encode the mitochondrial carrier family proteins that are involved in the transport of ornithine across the mitochondrial inner membrane [9]. The expression of *ORNT1* is high in liver. Expression of *ORNT1* in transformed fibroblasts of patients with HHH syndrome restored ornithine transport function. The gene contains eight exons over 26 kb (Camacho, personal communication, 2002). It encodes a 301 amino acid protein. A 4.2-kb mRNA transcript is expressed in liver and pancreas. The gene was mapped to chromosome 13q13-14 [9].

Among mutations observed [9,10], F188, a 3-bp inframe deletion in a sequence of four consecutive TTC phenylalanine codons encodes an unstable functionless protein. This mutation was found in nine of ten French-Canadian homozygotes and one heterozygote. E180K encodes a stable properly targeted protein and results from a G to A transition at bp 538. This mutation was found in the patient's Irish-American father, but not in his Japanese mother, who had a terminal microdeletion 13q14. Our patient [7] had a nonsense mutation R179ter (Camacho *et al.*, personal communication, 2002). A nonsense mutation R179X was found in two unrelated Japanese patients [16]. In eight Italian patients, nine different mutations were found, seven of them novel [33]. In a recent summary, 22 different mutations were listed for 49 patients in 31 unrelated families [19].

A second gene, *ORNT2*, has been discovered [34] which contains no introns and has a structure 88 percent identical to *ORNT1*. It is located on chromosome 5q31. Overexpression of protein product *ORNT2* in fibroblasts of patients with HHH syndrome restored the metabolism of ornithine, much as did *ORNT1*. The existence of this redundancy in ornithine transport was considered consistent with the generally milder phenotype in this disease than in other urea cycle abnormalities, as well as the level of residual ornithine transport observed in cultured F188 Δ and E180K cells. This conceptualization has been strengthened and amplified by the finding that the Mexican families with mild phenotype had a T32R mutation in *ORNT1*, but they also were heterozygous for the glycine 181 polymorphism in *ORNT2*, which is a gain of function variant.

A polymerase chain reaction (PCR)-based method for the detection of the F188 Δ mutation has been employed for newborn screening in Northern Saskatchewan, where HHH syndrome is found in high incidences [35]. This population is 94 percent Aboriginal and 66 percent French-Canadian Aboriginal, and it is isolated. Heterozygote frequency was in one in 1550 live births. Ornithine levels in the newborn period were normal in all three populations: mutant, carrier, and normal; thus, analysis for the gene is the only one that is useful for newborn screening.

TREATMENT

Restriction of the dietary intake of protein to 1.5 g/kg permitted maintenance of the blood ammonia at 90–100 mg/dL and the avoidance of acute attacks of hyperammonemia, whereas 2 g/kg led to symptomatic hyperammonemia [1]. In this patient, an acute load of 100 mg lysine/kg did not increase homocitrulline excretion. Supplementation of the diet with 1-g lysine hydrochloride increased its blood concentration to a low normal level, but did not appreciably change the plasma ornithine or ammonia in this 19-month-old patient. Supplementation with 1-g ornithine hydrochloride per day increased the

plasma concentration of ornithine, but had no effect on the postprandial ammonia or the plasma lysine. On the other hand, supplementation with 6 g/day of ornithine hydrochloride or 7.5 g of arginine hydrochloride in a 32–45-kg woman were reported to lower postprandial ammonia and urinary homocitrulline [12]. In this patient, supplementation with 6 g/day of lysine hydrochloride increased the excretion of homocitrulline. Ornithine supplementation reduced plasma concentrations of lysine. In another patient [36], supplementation with ornithine reduced the concentration of ammonia following a breakfast containing 0.5 g protein/kg from 273 to 107 mg/dL.

REFERENCES

- Shih VE, Effron ML, Moser HW. Hyperornithinemia hyperammonemia and homocitrullinemia. A new disorder of amino acid metabolism associated with myoclonic seizures and mental retardation. *Am J Dis Child* 1969; **117**: 83.
- Wright T, Pollitt R. Psychomotor retardation epileptic and stuporous attacks irritability and ataxia associated with ammonia intoxication high blood ornithine levels and increased homocitrulline in the urine. *Proc Royal Soc Med* 1973; **66**: 221.
- Gatfield PD, Taller E, Wolfe DM, Haust MD. Hyperornithinemia hyperammonemia and homocitrullinuria associated with decreased carbamyl phosphate synthetase I activity. *Pediatr Res* 1975; **9**: 488.
- Dionisi V, Bachmann C, Gambaram M *et al*. Hyperornithinemia-hyperammonemia-homocitrullinuria syndrome: low creatine excretion and effect of citrulline, arginine, or ornithine supplement. *Pediatr Res* 1987; **22**: 364.
- Inoue I, Shaheki T, Kayanuma K *et al*. Direct evidence of decreased ornithine transport activity in the liver mitochondria from patients with hyperornithinemia hyperammonemia and homocitrullinuria. In 4th International Congress of Inborn Errors of Metabolism, Sendai, Japan, May 26–30, 1987 (Abstr. FP-60).
- Hommes FA, Ho CK, Roesel RA, Coryell ME. Decreased transport of ornithine across the inner mitochondrial membrane as a cause of hyperornithinaemia. *J Inherit Metab Dis* 1982; **5**: 41.
- Nyhan WL, Rice-Asaro M, Acosta P. Advances in the treatment of amino acid and organic acid disorders. In: Desnick RJ (ed.). *Treatment of Genetic Diseases*. New York: Churchill Livingstone, 1991: 45.
- Oyanagi K, Tsuchiyama A, Itakura Y *et al*. The mechanism of hyperammonaemia and hyperornithinaemia in the syndrome of hyperornithinaemia hyperammonaemia and homocitrullinuria. *J Inherit Metab Dis* 1983; **6**: 133.
- Camacho JA, Obie C, Biery B *et al*. Hyperornithinaemia-hyperammonaemia-homocitrullinuria syndrome is caused by mutations in a gene encoding a mitochondrial ornithine transporter. *Nat Genet* 1999; **22**: 151.
- Camacho J, Mardach R, Rioseco-Camacho N *et al*. Phenotypic characterization and functional consequences of a mutation in the human mitochondrial ornithine transporter (ORNT1-T32R). *J Am Hum Genet* 2002; **71**(Suppl.): 418.
- Rodes M, Ribes A, Pineda M *et al*. A new family affected by the syndrome of hyperornithinaemia hyperammonaemia and homocitrullinuria. *J Inherit Metab Dis* 1987; **10**: 73.
- Winter HS, Perez-Atavde AR, Levy HL, Shih VE. Unique hepatic ultrastructural changes in a patient with hyperammonemia (HAM) hyperornithinemia (HOR) and homocitrullinuria (HC). *Pediatr Res* 1980; **14**: 583.
- Fell V, Pollitt R, Sampson GA, Trevor W. Ornithinemia hyperammonemia and homocitrullinuria. A disease associated with mental retardation and possibly caused by defective mitochondrial transport. *Am J Dis Child* 1974; **127**: 752.
- Koike R, Fujimori K, Yuasa T *et al*. Hyperornithinemia, hyperammonemia, and homocitrullinuria: case report and biochemical study. *Neurology* 1987; **37**: 1813.
- Miyamoto T, Kanazawa N, Kato S *et al*. Diagnosis of Japanese patients with HHH syndrome by molecular genetic analysis: a common mutation, R179X. *J Hum Genet* 2001; **46**: 260.
- Lemay JF, Lambert MA, Mitchell GA *et al*. Hyperammonemia-hyperornithinemia-homocitrullinuria syndrome: neurologic, ophthalmologic, and neuropsychologic examination of 6 patients. *J Pediatr* 1992; **121**: 725.
- Nakajima M, Ishli S, Mito T *et al*. Clinical, biochemical and ultrastructural study on the pathogenesis of hyperornithinemia-hyperammonemia-homocitrullinuria syndrome. *Brain Dev* 1988; **10**: 181.
- Smith L, Lambert MA, Brochu P *et al*. Hyperornithinemia, hyperammonemia, homocitrullinuria (HHH) syndrome: presentation as acute liver disease with coagulopathy. *J Pediatr Gastroenterol Nutr* 1992; **15**: 431.
- Debray FG, Lambert M, Lernieux B *et al*. Phenotypic variability among patients with hyperornithinemia-hyperammonemia-homocitrullinuria syndrome homozygous for the delF188 mutation in SLC25A15. *J Med Genet* 2008; **45**: 759.
- Wong P, Lessick M, Kang S *et al*. Maternal hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome. *Am J Hum Genet* 1989; **45**(Suppl.): A14.
- Oizumi J, Ng WG, Koch R *et al*. Partial ornithine transcarbamylase deficiency associated with recurrent hyperammonemia lethargy and depressed sensorium. *Clin Genet* 1984; **25**: 538.
- O'Donnell JJ, Sandman RP, Martin SR. Gyrate atrophy of the retina: inborn error of L-ornithine: 2-oxoacid aminotransferase. *Science* 1978; **200**: 200.
- Bickel H, Feist D, Muller H, Quadbeck G. Ornithinämie eine weitere Aminosäurenstoff-Wechselstörung mit Hirnschädigung. *Dtsch Med Wochenschr* 1968; **93**: 2247.
- Kekomaki MP, Raiha Niels CR, Bickel H. Ornithine-ketoacid aminotransferase in human liver with reference to patients with hyperornithinaemia and familial protein intolerance. *Clin Chem* 1969; **23**: 203.
- Gerritsen T, Waisman HA, Lipton SH, Strong FM. Natural occurrence of homocitrulline: I. Excretion in the urine. *Arch Biochem Biophys* 1962; **97**: 34.

26. Gerritsen T, Vaughn JG, Waisman HA. Origin of homocitrulline in the urine of infants. *Arch Biochem Biophys* 1963; **100**: 298.
27. Ryan WL, Wells IC. Homocitrulline and homoarginine synthesis from lysine. *Science* 1964; **144**: 1122.
28. Buergi W, Colombo JP, Richterich R. Thin-layer chromatography of the acid and ether soluble DNP-amino acids in urine. *Klin Wochenschr* 1965; **43**: 1202.
29. Oberholzer VG, Briddon A. 3-Amino-2-piperidone in the urine of patients with hyperornithinemia. *Clin Chim Acta* 1978; **87**: 411.
30. Fell V, Pollitt RJ. 3-Aminopiperid-2-one an unusual metabolite in the urine of a patient with hyperammonaemia hyperornithinaemia and homocitrullinuria. *Clin Chim Acta* 1978; **87**: 405.
31. Gamble JG, Lehninger AL. Transport of ornithine and citrulline across the mitochondrial membrane. *J Biol Chem* 1973; **248**: 610.
32. Shih VE, Mandell R, Herzfeld A. Defective ornithine metabolism in cultured skin fibroblasts from patients with the syndrome of hyperornithinemia hyperammonemia and homocitrullinuria. *Clin Chim Acta* 1982; **118**: 149.
33. Saivi S, Santorelli FM, Bertine E *et al*. Clinical and molecular findings in hyperornithinemia-hyperammonemia-homocitrullinuria syndrome. *Neurology* 2001; **57**: 911.
34. Camacho JA, Rioseco-Camacho N, Obie C *et al*. Cloning and characterization of ORNT2 a second mitochondrial ornithine transporter that can rescue a defective ORNT1 in patients with the hyperornithinemia-hyperammonemia-homocitrullinemia syndrome. A urea cycle disorder. *Mol Genet Metab* 2003; **79**: 257.
35. Sokoro AA, Lepage J, Antonishyn N *et al*. Diagnosis and high incidence of hyperornithinemia-hyperammonemia-homocitrullinemia (HHH) syndrome in northern Saskatchewan. *J Inher Metab Dis* 2010; June 24 [Epub ahead of print].
36. Gordon BA, Gatfield PD, Wolfe DM. Studies on the metabolic defect in patients with hyperammonemia hyperornithinemia and homocitrullinuria. *Clin Res* 1976; **24**: 688A.

Lysinuric protein intolerance

Introduction	235	Treatment	238
Clinical abnormalities	235	References	238
Genetics and pathogenesis	237		

MAJOR PHENOTYPIC EXPRESSION

Failure to thrive; episodic hyperammonemia; vomiting; diarrhea; pulmonary fibrosis and respiratory insufficiency; nephritis; low concentrations of lysine and other dibasic amino acids in plasma; and massive excretion of lysine in the urine along with increased excretion of ornithine and arginine; orotic aciduria; and decreased cellular transport of cationic amino acids, resulting from mutations in the gene *SLC7A7* for the amino acid transporter.

INTRODUCTION

Lysinuric protein intolerance was first described by Perheentupa and Visakorpi [1] from Finland in 1965 in a report of three patients with familial intolerance to protein and abnormal transport of the basic amino acids. The disease is prevalent in Finland, where it has been estimated to occur in one in 60,000 [2], and Finns or Finnish Lapps have comprised nearly half of the patients reported [1–6]. However, the disease may be found in any ethnic population. The fundamental defect is an abnormality in the transport of basic amino acids in the basilateral or antiluminal membrane of epithelial cells (Figure 30.1) [7–9]. The abnormality is in the efflux of these amino acids and can be demonstrated in cultured fibroblasts [10, 11]. The gene for the transporter has been mapped to chromosome 14q11.2 [12]. A founder mutation was found in the Finnish population [13] in the *SLC7A7* gene, a splice site acceptor change leading to a frame shift and a premature termination. More than 50 mutations have been found worldwide [14, 15].

CLINICAL ABNORMALITIES

Most infants present with failure to thrive (Figures 30.2, 30.3, and 30.4) [4]. There may be alopecia. Subcutaneous fat is diminished or absent, and the skin folds loose. Associated diarrhea may suggest a malabsorption

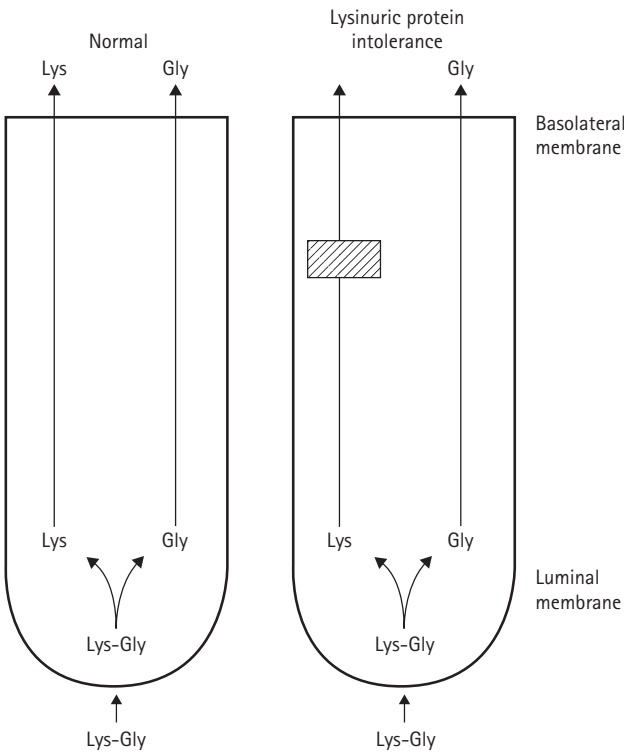


Figure 30.1 Demonstration of the site of the defect in lysinuric protein intolerance at the antiluminal border of the epithelial cell of the jejeunal mucosa by the administration of a lysylpeptide [6].



Figure 30.2 RQ: A patient with lysinuric protein intolerance who presented at five years of age with failure to thrive, alopecia, and skin lesions reminiscent of kwashiorkor or zinc deficiency. Treatment with citrulline reversed the findings in the skin and hair.



Figure 30.3 RQ: The perianal dermatitis was classic for a diagnosis of acrodermatitis enteropathica. Treatment of the failure to thrive with parenteral alimentation led to hyperammonemic coma. Levels of alanine and glutamine were elevated.

syndrome. Skin lesions may resemble those of kwashiorkor or acrodermatitis, and zinc deficiency (Figures 30.2 and 30.3). A dry scaly rash is sometimes seen, as well as sores on the sides of the mouth [16]. Dystrophic nails may contribute to the picture of acrodermatitis enteropathica (Figure 30.4). A five-year-old boy with chronic diarrhea and pitting edema of the lower extremities was thought to have celiac disease because villous atrophy was found on



Figure 30.4 RQ: The fingers and nails were also reminiscent of acrodermatitis enteropathica. The day after initiating citrulline, this bedridden patient was sitting up. She ultimately developed nails and hair.

intestinal biopsy [16]. There was no improvement with a gluten-free diet. There is usually some hepatomegaly. The spleen may be palpable. Body weight is reduced and linear growth falls off. Of 20 Finnish patients [6], 16 had heights that were 2–6 SDs below the mean. Head circumference is normal. Skeletal maturation is usually delayed. Anemia is the rule and leukopenia is common.

Osteoporosis develops in a majority of patients [17–19]. Two-thirds have had fractures, often after minimal trauma [3, 18–20]. Compression fractures of the vertebrae may lead to deformity. Fractures may occur before the age of five years. Metabolism of calcium and phosphate is normal, but hydroxyproline excretion in the urine is elevated.

Hyperammonemia is usually manifest as episodic attacks of vomiting. These may begin in neonatal infancy or be delayed even until adult life in patients with well-developed aversion to protein [6, 21]. Refusal to eat meat or dairy products has also been observed in patients who have not experienced hyperammonemia [14]. The avoidance of protein-containing foods is an early characteristic, which may begin as early as 12 months of age. Vomiting may be associated with dizziness or headaches. There may be loss of consciousness [22] or even deep coma and an isoelectric electroencephalograph (EEG), especially in patients administered large amounts of protein by gastric tube [6, 21, 23, 24]. Episodic psychiatric symptoms have been observed. Some patients have been mentally impaired, some severely so [25], but most are not. Nonprogressive, asymptomatic opacities have been observed in the lens of the eye [26].

A group of patients from southern Italy, in which consanguinity was high, has been described [27] with unusual complications. Manifestations in the patients included abnormalities of the bone marrow, in five of the six examined, in which large cells resembling sea-blue histiocytes suggested a diagnosis of Niemann-Pick disease, but sphingomyelinase was negative, and also there was

prominent erythrophagocytosis. Erythrophagocytosis and immunologic abnormalities were also described in another patient with this disease [28]. Duval *et al.* [29] have concluded that this is a regular feature of the disease and that it fits the diagnostic criteria for familial hemophagocytic lymphohistiocytosis (HPLH1). This hematologic picture has been seen along with the pancytopenia of propionic acidemia [30]. It has also been observed in carnitine palmitoyl transferase I deficiency [31] and in hemochromatosis [32]. Two patients had clinical pancreatitis [27]; in one who required surgery, pancreatic fibrosis was found, indicating chronic pancreatitis, as well as acute liponecrosis.

A number of late complications have been described. Some of these patients have presented first in adult life with interstitial disease of the lung or with renal disease.

Pulmonary disease has emerged as a major complication [27, 33, 34]. It has ranged from mild roentgenographic changes of fibrosis to severe interstitial infiltration, alveolar proteinosis, and death from respiratory failure. Respiratory insufficiency or clubbing may be the presenting complaint [34]. Cough, dyspnea, and hemoptysis may occur, and there may be intermittent fever or pulmonary infection. Roentgenograms show reticulonodular interstitial densities. Biopsy of the lung may show cholesterol crystals or granulomas [34] or alveolar proteinosis. Elevated concentrations of cationic amino acids in bronchoalveolar lavage fluid suggest that transport may also be abnormal in pulmonary epithelium [35]. In a number of patients with no pulmonary symptoms, there was roentgenographic evidence of pulmonary fibrosis [33].

Some patients have had chronic renal failure [27, 36] with proteinuria and progressive glomerular and tubular insufficiency. Disease consistent with systemic lupus erythematosus has been reported [37] and the pulmonary disease may be a manifestation of immune complex problems. A full Fanconi syndrome with clinical rickets and deformities may occur [27]. This may obscure the diagnosis because of a massive generalized aminoaciduria. Oral loading with lysine or arginine to test intestinal absorption may be required for diagnosis in such a patient.

Some children and some adults have developed terminal hepatic insufficiency. Pathology was that of extensive fatty degeneration and micronodular cirrhosis [38, 39]. In other patients, biopsy of the liver has been normal [4, 11] or there have been fat droplets in hepatocyte cytoplasm. At autopsy in the adult, changes were noted in the glomerular basement membrane, and immunofluorescence positive for IgA indicated an active glomerular lesion [39]. Terminal micronodular cirrhosis and pulmonary alveolar proteinosis were also found at autopsy.

GENETICS AND PATHOGENESIS

The disorder is inherited in an autosomal recessive pattern [2]. Many examples have been found of multiple affected individuals in a family [21, 22, 25, 36, 40–42].

The defect in amino acid transport is often first evident in the analysis of amino acids in the blood. Plasma concentrations of lysine, ornithine, and arginine are low. The mean concentration of lysine in 20 patients [43] was 70 mmol/L; those of ornithine and arginine were 21 and 27 mmol/L, respectively. The diagnosis may not be clear from these levels, because the normal ranges are that low. However, the plasma concentration of citrulline is impressively elevated. The mean was 232 mmol/L [43], which is about four times the upper limit of normal. Concentrations of glutamine and alanine are also elevated, consistent with chronic excess of ammonia. Concentrations of glycine, serine, and proline may also be elevated.

The urinary excretion of lysine is massively increased, and there is increased excretion of arginine and ornithine. The mean excretion of lysine per 1.73 m² body surface in 20 patients [43] was 4.13 mmol/24 hours; those of ornithine and arginine were 0.11 and 0.36 mmol, respectively. The renal clearance of lysine was 25.7 mL/min per 1.73 m²; those of ornithine and arginine were 3.3 and 11.5 mL/min per 1.73 m². Citrulline and glutamine are excreted in large amounts, but their clearance is normal. Defective renal tubular reabsorption has been demonstrated and is most marked in the case of lysine, less so for arginine, and least for ornithine [44]. The abnormal pattern of urinary amino acids may be elusive, especially when quantification is not employed, at times of very low plasma levels of the basic amino acids and rigid restriction of the dietary intake of protein. Increasing the plasma concentration of lysine clarifies the diagnosis.

Absorption of basic amino acids is defective in the small intestine [6, 45–47], as well as in the renal tubule. In an interesting assessment of mechanism, the oral administration of lysylglycine to patients led to an increase in plasma concentrations of glycine, but not of lysine, while in controls both increased [6]. Thus, the lysine dipeptide was normally absorbed by patients across the luminal membrane and hydrolyzed intracellularly, but efflux of the lysine, though not of the glycine, was blocked at the antiluminal membrane. *In vitro* studies of biopsied jejunum confirmed this position of the defect [8]. This is very different from cystinuria, where the defect is in the luminal membrane.

The defective transport in lysinuric protein intolerance is expressed in cultured fibroblasts [9]. Labeled lysine and the nonmetabolizable analog, homoarginine [47], were found not to display the trans-stimulated efflux that occurs in the presence of a cationic amino acid on the other side of the membrane [9, 48, 49]. Heterozygotes were found to display approximately 50 percent of control activity [9].

The mechanisms by which hyperammonemia occurs are not completely understood. Concentrations of ammonia are normal in the fasting state, but increase up to 500 μ molar postprandially [4, 11]. Persistent hyperammonemia may result from a large protein intake, prolonged fasting, or infection. The urinary excretion of orotic acid is usually elevated in patients [50, 51] even when they are receiving

diets restricted in protein, and there is a major increase after the administration of a protein or alanine load [51]. The concentration of urea in these patients is usually low.

Abnormal function of the urea cycle is thought to result from intramitochondrial shortage of ornithine, as in the hyperornithinemia, hyperammonemia, homocitrullinuria (HHH) syndrome (Chapter 30). In lysinuric protein intolerance, intravenous infusion of ornithine or arginine (an ornithine source) prevents the hyperammonemia of protein or alanine loading [1, 4, 11]. Infusion of citrulline also accomplishes this effect and, furthermore, it is effective orally [46]. Oral arginine and ornithine are less effective because they are so poorly absorbed in this condition that supplementation leads to diarrhea [8, 47]. Citrulline is a neutral amino acid and is absorbed via a different transport system, but once in the cell, it is converted via arginine to ornithine. The nature of the defect in efflux would make for high intracellular concentration of ornithine, but the trans-stimulated system has not been found in hepatocytes [52, 53], so hepatic cells would be expected to reflect the depletion of dibasic amino acids evident in the plasma. Some clinical manifestations, such as failure to thrive, anemia, hepatomegaly, and osteoporosis, could be a function of a shortage of lysine.

The molecular locus for lysinuric protein intolerance was assigned to chromosome 14q11.2 in a study of 20 Finnish families [12]. The gene for the carrier protein, SLC7A7 (solute carrier family 7, member 7) maps to this site, and a search for mutations in the Finnish families yielded a mutant allele (1181-2A>T) in which an A to T transversion at 22 of the acceptor splice site in intron 6 leads to altered splicing deleting 10 base pairs and a frame shift [13]. A common haplotype was consistent with a single founder mutation. This mutation was found independently by Italian investigators [54] who also found a frame shift mutation in Italian patients resulting from homozygosity for a 4-bp insertion (1625 ins ATAC). A 543-bp deletion was found in another Italian proband. Among other mutations identified [14, 15, 55, 56]. Expression studies of mutations have revealed proteins that failed to localize to the plasma membrane, as well as proteins that localized but failed to function. Quite different clinical phenotypes have been observed in individuals with the same genotype and genotype-phenotype correlations have been elusive [14], but large deletions involving exons 4–11 and 6–11 had very severe clinical phenotypes [15].

TREATMENT

The effects of citrulline in this condition have formed the basis for effective therapy [5, 11]. Citrulline is provided in doses of 2.5–8.5 g daily, usually divided into three to five doses, especially with meals. Citrulline supplementation produces an adequate quantity of urea cycle intermediates, and in this way prevents hyperammonemia. Protein intake is moderately restricted, a process most patients have begun

spontaneously. Intakes of 1–1.5 g/kg per day in children and 0.5–0.7 g/kg in adults have been employed.

In an acute crisis of hyperammonemia, protein intake is stopped, and energy is supplied as intravenous glucose. Infusion of arginine, ornithine, or citrulline should be effective. Dosage recommended has been 1 mmol/kg as a primary dose followed by 0.5–1 mmol/kg per hour until symptoms are eliminated. Intravenous sodium benzoate or phenylacetate, or both, may be employed as adjunctive therapy [57].

Lysine depletion may be improved with supplemental lysine [58], but this is limited by malabsorption and intestinal tolerance. ϵ -N-Acetyllysine has been shown to increase plasma concentrations of lysine [59]. Increase may also be accomplished by the intravenous administration of lysine [60].

Pulmonary disease may be effectively treated with high-dose regimens of prednisolone [43], but some patients have not responded. Successful treatment of renal complications has not been reported.

Elevated levels of cholesterol and triglyceride were documented in 39 Finnish patients [61] whose fat intake was no higher than the general population. Successful lowering was obtained with statin therapy. The authors recommended atorvastatin over others because of more effective reduction of both cholesterol and triglyceride.

REFERENCES

1. Perheentupa J, Visakorpi JK. Protein intolerance with deficient transport of basic amino acids. *Lancet* 1965; **2**: 813.
2. Norio R, Perheentupa J, Kekomäki M, Visakorpi JK. Lysinuric protein intolerance an autosomal recessive disease. *Clin Genet* 1971; **2**: 214.
3. Simell O, Rajantie J, Perheentupa J. Lysinuric protein intolerance. In: Eriksson AW, Forsius H, Nevanlinna HR *et al.* (eds). *Population Structure and Genetic Disorders*. London: Academic Press, 1980: 633.
4. Kekomäki M, Visakorpi JK, Perheentupa J, Saxen L. Familial protein intolerance with deficient transport of basic amino acids. An analysis of 10 patients. *Acta Paediatr Scand* 1967; **56L**: 617.
5. Awrich AE, Stackhouse J, Cantrell JE *et al.* Hyperdibasic aminoaciduria hyperammonemia and growth retardation: treatment with arginine lysine and citrulline. *J Pediatr* 1975; **8**: 731.
6. Simell O, Perheentupa J, Rapola J *et al.* Lysinuric protein intolerance. *Am J Med* 1975; **59**: 229.
7. Rajantie J, Simell O, Perheentupa J. Basolateral-membrane transport defect for lysine in lysinuric protein intolerance. *Lancet* 1980; **1**: 1219.
8. Rajantie J, Simell O, Perheentupa J. Lysinuric protein intolerance. Basolateral transport defect in renal tubuli. *J Clin Invest* 1981; **67**: 1078.
9. Desjeux JF, Rajantie J, Simell O *et al.* Lysine fluxes across the

- jejunal epithelium in lysinuric protein intolerance. *J Clin Invest* 1980; **65**: 1382.
10. Smith DW, Scriver CR, Tenenhouse HS, Simell O. Lysinuric protein intolerance mutation is expressed in the plasma membrane of cultured skin fibroblasts. *Proc Natl Acad Sci USA* 1987; **84**: 7711.
11. Botschner J, Smith DW, Simell O, Scriver CR. Comparison of ornithine metabolism in hyperornithinemia-hyperammonemia-homocitrullinuria syndrome lysinuric protein intolerance and gyrate atrophy fibroblasts. *J Inherit Metab Dis* 1989; **12**: 33.
12. Lauteala T, Sistonen P, Savontaus M-L et al. Lysinuric protein intolerance (LPI) gene maps to the long arm of chromosome 14. *Am J Hum Genet* 1997; **60**: 1479.
13. Torrents D, Mykkänen J, Pineda M et al. Identification of SLC7A7 encoding yLAT-1 as the lysinuric protein intolerance gene. *Nat Genet* 1999; **21**: 293.
14. Sperandio MP, Andria G, Sebastio G. Lysinuric protein intolerance: update and extended mutation analysis of the SLC7A7 gene. *Hum Mutat* 2008; **29**: 14.
15. Font-Llitjos M, Rodriguez-Santiago B, Espino M et al. Novel SLC7A7 large rearrangements in lysinuric protein intolerance patients involving the same AluY repeat. *Eur J Hum Genet* 2009; **17**: 71.
16. Reinoso MA, Whitley C, Jessurun J, Schwarzenberg SJ. Lysinuric protein intolerance masquerading as celiac disease: a case report. *J Pediatr* 1998; **132**: 153.
17. Svedström E, Parto K, Marttinen M et al. Skeletal manifestations of lysinuric protein intolerance. *Skeletal Radiol* 1993; **22**: 11.
18. Parto K, Penttinen R, Paronen I et al. Osteoporosis in lysinuric protein intolerance. *J Inherit Metab Dis* 1993; **16**: 441.
19. Carpenter TO, Levy HL, Holtrop ME et al. Lysinuric protein intolerance presenting as childhood osteoporosis. Clinical and skeletal response to citrulline therapy. *N Engl J Med* 1985; **312**: 290.
20. Mori H, Kimura M, Fukuda S. A case of lysinuric protein intolerance with mental-physical retardation intermittent stupor and hemiparesis. *Rinsho Shinkeigaku* 1982; **22**: 42.
21. Shaw PJ, Dale G, Bates D. Familial lysinuric protein intolerance presenting as coma in two adult siblings. *J Neurol Neurosurg Psychiatry* 1989; **52**: 648.
22. Yoshimura T, Kato M, Goto I, Kuroiwa Y. Lysinuric protein intolerance – two patients in a family with loss of consciousness and growth retardation. *Rinsho Shinkeigaku* 1983; **23**: 140.
23. Chan H, Billmeier GJ Jr, Molinary SV et al. Prolonged coma and isoelectric electroencephalogram in a child with lysinuric protein intolerance. *J Pediatr* 1977; **91**: 79.
24. Coude FX, Ogier H, Charpentier C et al. Lysinuric protein intolerance: a severe hyperammonemia secondary to L-arginine deficiency. *Arch Fr Pediatr* 1981; **38**(Suppl. 1): 829.
25. Oyanagi K, Miuyra R, Yamanouchi T. Congenital lysinuria: a new inherited transport disorder of dibasic amino acids. *J Pediatr* 1970; **77**: 259.
26. Moschos M, Andreanos D. Lysinuria and changes in the crystalline lens. *Bull Mem Soc Fr Ophthalmol* 1985; **96**: 322.
27. Parenti G, Sebastio G, Strisciuglio P et al. Lysinuric protein intolerance characterized by bone marrow abnormalities and severe clinical course. *J Pediatr* 1995; **126**: 246.
28. Gursel T, Kocak U, Tumer L, Hasanoglu A. Bone marrow hemophagocytosis and immunological abnormalities in a patient with lysinuric protein intolerance. *Acta Hematologica* 1997; **98**: 160.
29. Duval M, Fenneteau O, Doireau V et al. Intermittent hemophagocytic lymphohistiocytosis is a regular feature of lysinuric protein intolerance. *J Pediatr* 1999; **134**: 236.
30. Stork LC, Ambruso DR, Wallner SF et al. Pancytopenia in propionic acidemia: hematologic evaluation and studies of hematopoiesis *in vitro*. *Pediatr Res* 1986; **20**: 783.
31. Al Aqeel AI, Rashed MS, Ijst L et al. Phenotypic variability of carnitine palmitoyl transferase I deficiency (CPT I) with novel molecular defect in Saudi Arabia. *Am J Hum Genet* 2002; **71**: 412.
32. Parizhskaya M, Reyes J, Jaffe R. Hemophagocytic syndrome presenting as acute hepatic failure in two infants: clinical overlap with neonatal hemochromatosis. *Pediatr Dev Pathol* 1999; **2**: 360.
33. Parto K, Svedstrom E, Majurin ML et al. Pulmonary manifestations in lysinuric protein intolerance. *Chest* 1993; **104**: 1176.
34. Kerem E, Elpeg ON, Shalev RS et al. Lysinuric protein intolerance with chronic interstitial lung disease and pulmonary cholesterol granulomas at onset. *J Pediatr* 1993; **123**: 275.
35. Hallman M, Maasilta P, Sipilä I, Tahvanainen J. Composition and function of pulmonary surfactant in adult respiratory distress syndrome. *Eur Respir J* 1989; **2**(Suppl. 3): 104.
36. DiRocco M, Garibotto G, Rossi GA et al. Role of haematological pulmonary and renal complications in the long-term prognosis of patients with lysinuric protein intolerance. *Eur J Pediatr* 1993; **152**: 437.
37. Parsons H, Snyder F, Bowen T et al. Immune complex disease consistent with systemic lupus erythematosus in a patient with lysinuric protein intolerance. *J Inherit Metab Dis* 1996; **19**: 627.
38. Sidransky H, Verney E. Chemical pathology of diamino acid deficiency: considerations in relation to lysinuric protein intolerance. *J Exp Pathol* 1985; **2**: 47.
39. Moore R, McManus DT, Rodgers C et al. Lysinuric protein intolerance. *Proc SSIEM* 1995; **33**: 62 (Abstr. P050).
40. McManus DT, Moore R, Hill CM et al. Necropsy findings in lysinuric protein intolerance. *J Clin Pathol* 1996; **49**: 345.
41. Carson NAJ, Redmond OAB. Lysinuric protein intolerance. *Ann Clin Biochem* 1977; **14**: 135.
42. Kato T, Mizutani N, Ban M. Hyperammonemia in lysinuric protein intolerance. *Pediatrics* 1984; **73**: 489.
43. Simell O. Lysinuric protein intolerance and other cationic aminoacidurias. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic and Molecular Basis of Inherited Disease*. New York: McGraw Hill, 2001: 4933.
44. Simell O, Perheentupa J. Renal handling of diamino acids in lysinuric protein intolerance. *J Clin Invest* 1974; **54**: 9.
45. Kekamäki M. Intestinal absorption of L-arginine and L-lysine in familial protein intolerance. *Ann Paediatr Fenn* 1968; **14**: 18.

46. Rajantie J, Simell O, Perheentupa J. Oral administration of urea cycle intermediates in lysinuric protein intolerance: effect on plasma and urine arginine and ornithine. *Metabolism* 1983; **32**: 49.
47. Rajantie J, Simell O, Perheentupa J. Intestinal absorption in lysinuric protein intolerance: impaired for diamino acids normal for citrulline. *Gut* 1980; **21**: 519.
48. Christensen HN, Cullen AM. Synthesis of metabolism-resistant substrates for the transport system for cationic amino acids; their stimulation of the release of insulin and glucagon and of the urinary loss of amino acids related to cystinuria. *Biochim Biophys Acta* 1973; **298**: 932.
49. Rajantie J, Simell O, Perheentupa J. 'Basolateral' and mitochondrial membrane transport defect in the hepatocytes in lysinuric protein intolerance. *Acta Paediatr Scand* 1983; **72**: 65.
50. Sanjurjo Crespo P, Vallo Boado A, Prats-Viñas JM *et al*. Intolerancia proteica con lisinuria (aciduria dibásica). A propósito de un caso. *An Esp Pediatr* 1995; **42**: 219.
51. Rajantie J. Orotic aciduria in lysinuric protein intolerance: dependence on the urea cycle intermediates. *Pediatr Res* 1981; **15**: 115.
52. White MF, Christensen HN. Cationic amino acid transport into cultured animal cells. II. Transport system barely perceptible in ordinary hepatocytes but active in hepatoma cell lines. *J Biol Chem* 1982; **257**: 4450.
53. Christensen HN. Role of amino acid transport and countertransport in nutrition and metabolism. *Physiol Rev* 1990; **70**: 43.
54. Borsani G, Bassi MT, Sperandeo MP *et al*. SLC7A7 encoding a putative permease-related protein is mutated in patients with lysinuric protein intolerance. *Nat Genet* 1999; **21**: 297.
55. Mykkänen J, Torrents D, Pineda M *et al*. Functional analysis of novel mutations in y1LAT-1 amino acid transporter gene causing lysinuric protein intolerance (LPI). *Hum Mol Genet* 2000; **9**: 431.
56. Sperandeo MP, Bassi MT, Riboni M *et al*. Structure of the SLC7A7 gene and mutational analysis of patients affected by lysinuric protein intolerance. *Am J Hum Genet* 2000; **66**: 92.
57. Simell O, Sipilä I, Rajantie J *et al*. Waste nitrogen excretion via amino acid acylation: benzoate and phenyl-acetate in lysinuric protein intolerance. *Pediatr Res* 1986; **20**: 1117.
58. Rajantie J, Simell O, Rapola J, Perheentupa J. Lysinuric protein I intolerance: a two-year trial of dietary supplementation therapy with citrulline and lysine. *J Pediatr* 1980; **97**: 927.
59. Rajantie J, Simell O, Perheentupa J. Oral administration of ε-N-acetyllysine and homocitrulline for lysinuric protein intolerance. *J Pediatr* 1983; **102**: 388.
60. Lukkariinen MJ, Nanto-Salonen KM, Pulkki KJ *et al*. Lysine loading test in LPI-patients. *Proc SSIEM* 1995; **33**: 62 (Abstr. P051).
61. Tanner L, Niinikoski H, Nanto-Salonen K, Simell O. Combined hyperlipidemia in patients with lysinuric protein intolerance. *J Inherit Metab Dis* 2010; Feb 23 [Epub ahead of print].

Glutamine synthetase deficiency

Introduction	241	Treatment	243
Clinical abnormalities	241	References	243
Genetics and pathogenesis	243		

MAJOR PHENOTYPIC EXPRESSION

Prenatal onset malformations of the brain, seizures, necrolytic erythema of the skin, enteropathy with diarrhea, early neonatal death, and multiorgan failure or chronic encephalopathy and developmental delay, hyperammonemia, low concentration of glutamine in plasma and cerebrospinal fluid, and deficient activity of glutamine synthetase.

INTRODUCTION

Congenital deficiency of glutamine synthetase was first described by Häberle and colleagues in 2005 [1, 2] in two unrelated newborn infants. Each was the product of consanguineous Turkish parents. They died at 2 days and 4 weeks of life, respectively. One had micromelia and both had malformations of the brain. Thus, this disease can be added to those inborn errors of metabolism that express prenatally with congenital malformation syndromes. The disease can also be added to the list of rare disorders that lead to defective synthesis of an amino acid.

Concentrations of glutamine were low in plasma, urine, and cerebrospinal fluid. Concentrations of ammonia were moderately elevated. Deficient activity of glutamine synthetase (Figure 31.1) was found in immortalized lymphocytes derived from the initial patient [2]. Each infant was homozygous for an arginine to cysteine substitution in exon 6, p.R324C and p.R341C. A third patient has been reported [3] with a somewhat more attenuated course who had a p.R324S mutation.

CLINICAL ABNORMALITIES

The first patient had to be resuscitated at birth, required ventilator support, was flaccid, and showed no signs of development. He died of cardiac arrest at 2 days of life. The second patient had convulsions and respiratory failure requiring mechanical ventilation. She had voluminous stools and lost weight with enteral feeding; parental nutrition was instituted, but she developed hyponatremia. The third patient was alive at four years of age, but continued to have chronic encephalopathy and seizures.

The first patient had polyhydramnios. Congenital malformations included micromelia. Head circumference at 34 cm was in the 75th–90th percentile, while length and weight were in the 3rd–10th percentile. There were flexion contractures at the elbows and knees, camptodactyly, ulnar deviation of the hands, anteverted nostrils, and thin lips. Both initial patients had a flat or broad nasal roots and low set ears.

The second patient developed an erythematous rash at 2 weeks that became blistering after a few days (Figure

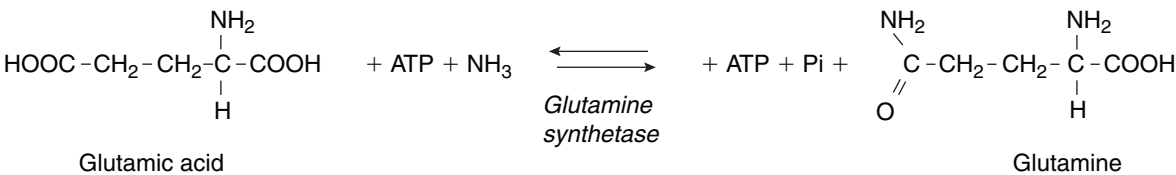


Figure 31.1 The glutamine synthetase reaction.



Figure 31.2 The necrolytic erythema of severe glutamine synthetase deficiency in the second patient described [1, 2]. Illustration was kindly provided by Dr Johannes Häberle, Universitäts-Kinderspital, Zurich.

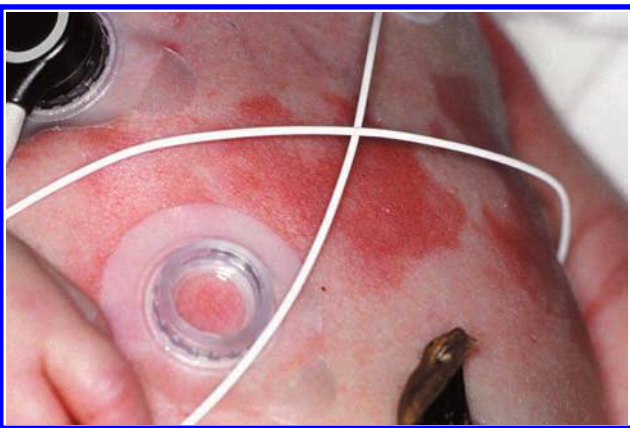


Figure 31.3 Closer view of the necrolytic erythema on the trunk of this patient. Illustration was kindly provided by Dr Johannes Häberle, Universitäts-Kinderspital, Zurich.



Figure 31.4 Necrolytic erythema in genital region in of the same patient. Illustration was kindly provided by Dr Johannes Häberle, Universitäts-Kinderspital, Zurich.



Figure 31.5 Necrolytic erythema in the third patient at three years of age. Illustration was kindly provided by Dr Johannes Häberle, Universitäts-Kinderspital, Zurich.

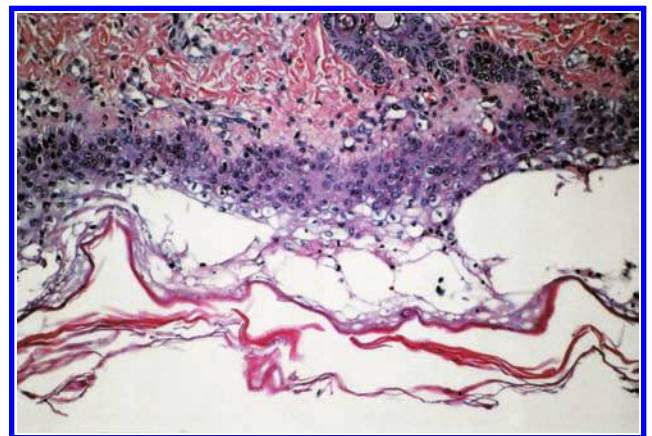


Figure 31.6 Histologic appearance of necrolytic erythema in severe glutamine synthetase deficiency. There was swelling of keratinocytes with condensed nuclei and intraepidermal blistering. Illustration was kindly provided by Dr Johannes Häberle, Universitäts-Kinderspital, Zurich.

31.2, 31.3, and 31.4). It became quite generalized. The third patient also developed necrolytic erythema (Figure 31.5). Histologic examination of the skin revealed intraepidermal blistering (Figure 31.6).

Electroencephalogram (EEG) in patient one showed very little cerebral activity and short bursts of theta waves and generalized seizures. Magnetic resonance imaging (MRI) of the brain of the first patient revealed delayed myelination, poor gyration, and large subependymal cysts (Figure 31.7). In the second patient, there was also delayed myelination and poor gyration, as well as subependymal cysts (Figure 31.8).

Patients with this disease have moderate hyperammonemia. Levels ranged from 140 to 400 $\mu\text{mol/L}$ (normal <110). Concentrations of glutamine in plasma were low in all three patients. Levels of 2 and 6 $\mu\text{mol/L}$ were

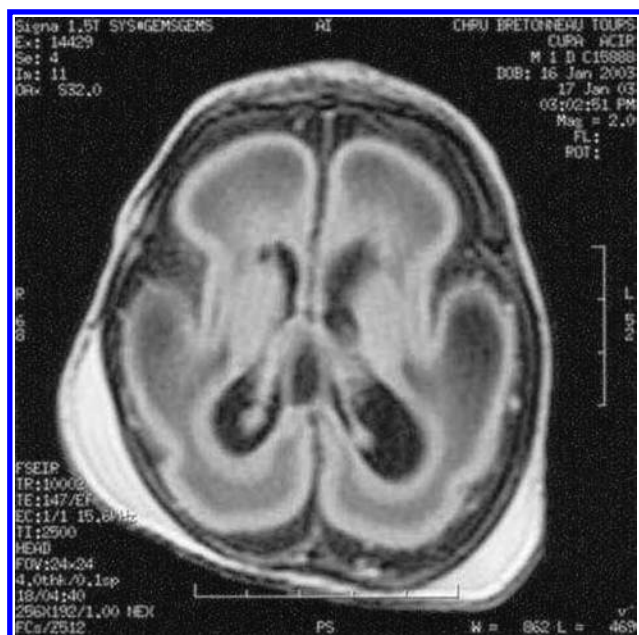


Figure 31.7 T₁-weighted in magnetic resonance imaging of the brain of the first patient illustrating severely delayed myelination and gyration and large subependymal cysts. Illustration was kindly provided by Dr Johannes Häberle, Universitäts-Kinderspital, Zurich.

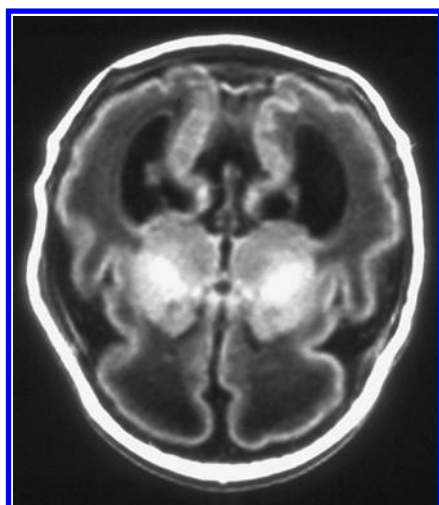


Figure 31.8 T₁-weighted magnetic resonance imaging of the brain of the second patient illustrating delayed myelination and gyration, and subependymal cysts. Illustration was kindly provided by Dr Johannes Häberle, Universitäts-Kinderspital, Zurich.

recorded (normal, 300–800) [1]. In urine, concentrations ranged from undetectable to 8 µmol/g creatinine (normal, 52–3230). Concentrations in the cerebral spinal fluid were 11 and 12 µmol/L (normal, 352–1280).

GENETICS AND PATHOGENESIS

The disease is autosomal recessive. So far, each of the families has been consanguineous, the first two Turkish and the third Arabs from Sudan.

The gene *GLUL*, which codes for glutamine synthetase (glutamate-ammonia ligase), consists of six exons [1] over 1122 bp and encodes a 374 amino acid polypeptide with a mass of 42 kDa [1, 4]. The enzyme is mitochondrial [5]. The gene has been mapped to chromosome 1,1q31 [6, 7]. There is a pseudogene on chromosome 9.

The mutations discovered to date represent a small area of exon 6, and all three involve arginine; two of them R324 and the other R341. In two, the arginine was converted to cysteine, and the other R324 was converted to serine.

Glutamine synthesis is involved in ammonia utilization, nitrogen flux, and the regulation of acid-base balance. The enzyme is abundantly active in brain, liver, and muscle [8, 9]. Fetal requirements for glutamine are quite high [10]. In brain, glutamine synthetase is found predominantly in astrocytes [11]. In the nervous system, glutamine is thought to be neuroprotective through lowering levels of both glutamate, which is excitotoxic, and ammonia. The synthetase is the only enzyme in man capable of synthesis of glutamine; without it, glutamine becomes an essential amino acid.

TREATMENT

Treatment has been supportive. Seizures require anti-convulsant therapy.

REFERENCES

1. Häberle J, Görg B, Rutsch F *et al.* Congenital glutamine deficiency with glutamine synthetase mutations. *N Engl J Med* 2005; **3**: 353.
2. Häberle J, Görg B, Toutain A *et al.* Inborn error of amino acid synthesis: human glutamine synthetase deficiency. *J Inherit Metab Dis* 2006; **29**: 352.
3. Häberle J, Shahbeck N, Ibrahim K *et al.* Natural course of glutamine synthetase deficiency in a 3 year old patient. *Mol Genet Metab* 2011; **103**: 89.
4. Gibbs CS, Campbell KE, Wilson RH. Sequence of a human glutamine synthetase cDNA. *Nucleic Acids Res* 1987; **15**: 6293.
5. Pesole G, Bozzetti MP, Lanave C *et al.* Glutamine synthetase gene evolution: a good molecular clock. *Proc Natl Acad Sci USA* 1991; **88**: 522.
6. Clancy KP, Berger R, Cox M *et al.* Localization of the L-glutamine synthetase gene to chromosome 1q23. *Genomics* 1996; **38**: 418.
7. Kolker S, Hoffmann GF, Okun JG. Comment on congenital glutamine deficiency with glutamine synthetase mutations. *N Engl J Med* 2006; **354**: 1094.

8. Häussinger D. Hepatic glutamine transport and metabolism. *Adv Enzymol Relat Areas Mol Biol* 1998; **72**: 43.
9. Suarez I, Bodega G, Fernandez B. Glutamine synthetase in brain: effect of ammonia. *Neurochem Int* 2002; **41**: 123.
10. Self JT, Spencer TE, Johnson GA *et al*. Glutamine synthesis in the developing porcine placenta. *Biol Reprod* 2004; **70**: 1444.
11. Martinez-Hernandez A, Bell KP, Norenberg MD. Glutamine synthetase: glial localization in brain. *Science* 1977; **195**: 1356.

DISORDERS OF FATTY ACID OXIDATION

32.	Introduction to disorders of fatty acid oxidation	247
33.	Carnitine transporter deficiency	253
34.	Carnitine-acylcarnitine translocase deficiency	260
35.	Carnitine palmitoyl transferase I deficiency	267
36.	Carnitine palmitoyl transferase II deficiency, lethal neonatal	273
37.	Carnitine palmitoyl transferase II deficiency, late onset	277
38.	Medium chain acyl CoA dehydrogenase deficiency	281
39.	Very long chain acyl CoA dehydrogenase deficiency	289
40.	Long chain L-3-hydroxyacyl CoA dehydrogenase – (trifunctional protein deficiency)	295
41.	Short-chain acyl CoA dehydrogenase deficiency	302
42.	3-HydroxyacylCoA dehydrogenase (short-chain 3-hydroxyacylCoA dehydrogenase) deficiency	309
43.	Short/branched chain acyl-CoA dehydrogenase (2-methylbutyrylCoA dehydrogenase) deficiency	312
44.	Multiple acyl CoA dehydrogenase deficiency/glutaric aciduria type II/ethylmalonic-adipic aciduria	316
45.	3-Hydroxy-3-methylglutarylCoA lyase deficiency	325

Introduction to disorders of fatty acid oxidation

The genetically determined disorders of fatty acid oxidation represent a recently rapidly growing group of inborn errors of metabolism. The field, as we know it today, really dates from the discovery in 1982 of medium-chain acyl CoA dehydrogenase (MCAD) deficiency ([Chapter 38](#)) [1, 2]. Myopathic carnitine palmitoyl transferase (CPTII) ([Chapter 37](#)) deficiency was known for some time earlier, but considered among myopathies not a forerunner of expansive growth of knowledge, and HMG CoA lyase deficiency ([Chapter 45](#)) had been described, but considered to be an organic acidemia. Multiple acyl CoA dehydrogenase deficiency ([Chapter 44](#)) was also known since 1976. The fact that MCAD deficiency turned out to be common and largely the consequence of a single mutation, has contributed to the current recognition of the importance of this group of disorders. In subsequent years, the rates of discovery of previously unrecognized disorders of fatty acid oxidation was exponential. The advent of diagnosis by tandem mass spectrometry and its application to programs of expanded screening of newborns [3] have opened up this entire population to the prevention of death and disability.

A summation of the various pathways involved in fatty acid oxidation and their interrelations is shown in [Figure 32.1](#). Abnormality in those pathways has often first been suggested chemically by the excretion of dicarboxylic acids in the urine. Dicarboxylic aciduria may also be dietary, especially in infants receiving formulas containing medium-chain triglycerides. When β -oxidation is defective, ω -oxidation and hydroxylation take place in the microsomal P450 system. This takes place efficiently in the case of long-chain fatty acyl CoA compounds, but the affinity of the system for medium-chain chain compounds is so low that they are thought to result from β -oxidation in peroxisomes of longer chain dicarboxylic or hydroxy acids [4].

Disorders of fatty acid oxidation may present with myopathy or cardiomyopathy. They may also present with sudden infant death syndrome (SIDS), but often the initial presentation is with a Reye-like episode of hypoketotic hypoglycemia, often with elevated blood concentrations of creatine kinase (CK), and uric acid, as well as transaminases [5]. Thus, in a hypoglycemic infant or child, evaluation of uric acid and CK (neither of which are routinely included

in metabolic clinical chemistry panels in children's hospitals), serves as an alerting signal to the presence of a disorder of fatty acid oxidation. In a hypoglycemic patient, the absence of ketones in the urine signifies that it is hypoketotic. However, the presence of ketones in the urine may be misleading. Blood levels of free fatty acids and 3-hydroxybutyrate may be required to make this distinction. Hyperammonemia may be seen at the time of the acute episode, and this may lead to a diagnosis of Reye syndrome. Actually, most patients that we see today with Reye syndrome have a disorder of fatty acid oxidation, even if a liver biopsy appearance of microvesicular fat appears typical of Reye syndrome. A few such patients have a urea cycle defect, but we have seen orotic aciduria, the hallmark of ornithine transcarbamylase deficiency ([Chapter 24](#)) in a patient with MCAD deficiency.

We have developed a systematic algorithmic approach to the work up of such a patient ([Figure 32.2](#)). The patient is often referred after the initial episode has been treated with glucose and fluids, and examination of the urine is negative except in the case of HMG CoA lyase deficiency. Modern work up begins with the assay of the DNA for the A985G mutation in the MCAD gene or tandem mass spectrometry for acylcarnitines, or both. Study of blood and urine concentrations of carnitine and its ester fraction may point to the answer. In some patients, a controlled but prolonged fast is necessary to elucidate the nature of the defect, but this is less true since the availability of acylcarnitine profiles and mutational analysis for the common MCAD mutation.

The normal response to fasting and the oxidation of fat begins with lipolysis, which releases free fatty acids. In patients with disorders of fatty acid oxidation, concentrations of free fatty acids are usually higher than those of 3-hydroxybutyrate in blood at times of illness and metabolic stress. Thus, assessment of the concentrations of free fatty acids and 3-hydroxybutyrate in the blood is essential to the diagnosis of hypoketosis. Because fatty acids that accumulate in the presence of defective oxidation undergo ω -oxidation to dicarboxylic acids, a disproportionate ratio of dicarboxylic acids to 3-hydroxybutyrate in the organic acid analysis of the urine also indicates disordered fatty acid oxidation. Transport of long-chain fatty acids into the mitochondria,

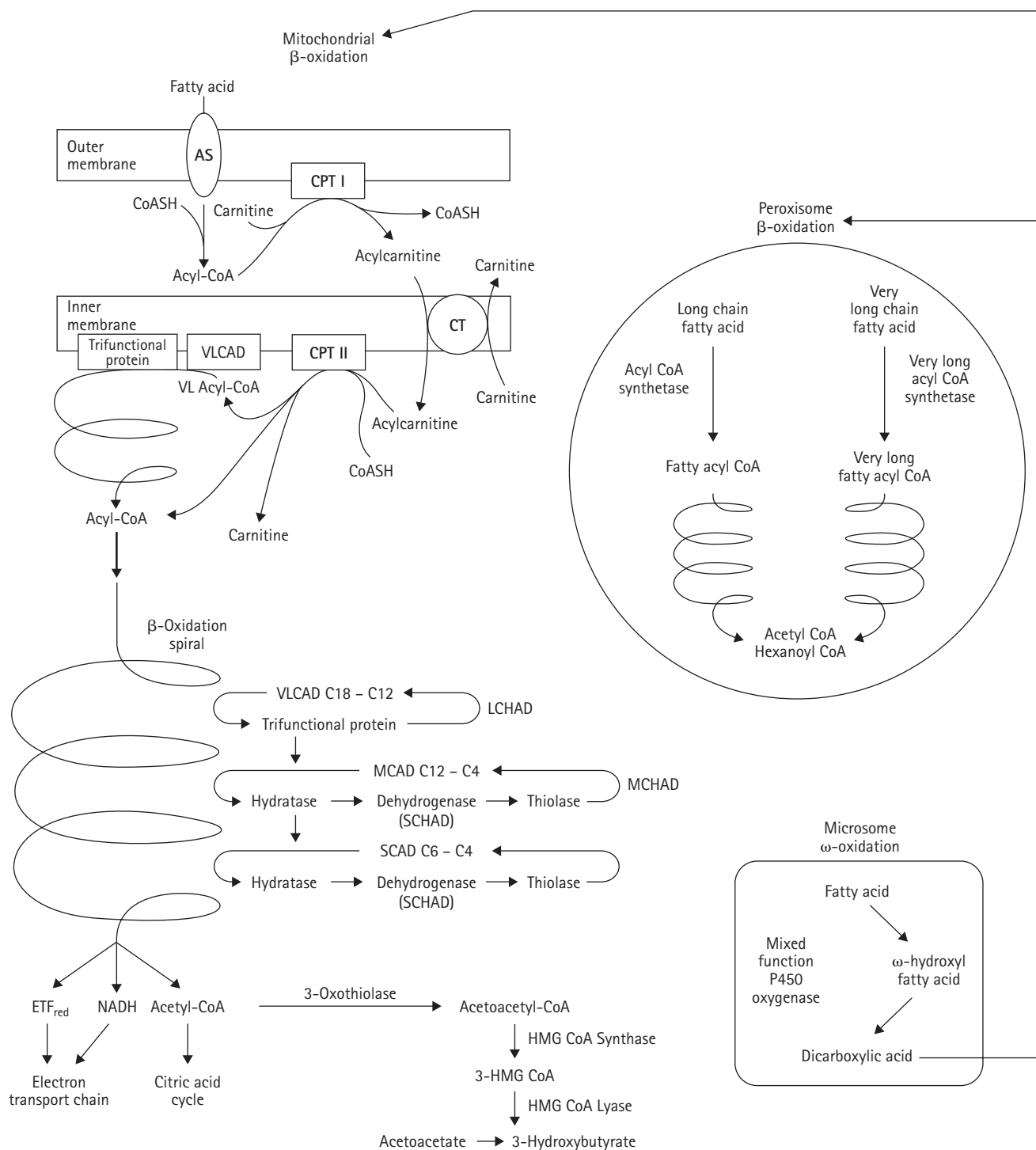


Figure 32.1 Metabolic pathways of fatty acid oxidation. Mitochondrial activities are central, but peroxisomal and microsomal oxidation also play a role.

where β -oxidation takes place, requires carnitine, and the entry of carnitine into cells, such as muscle, requires a specific transporter, which may be deficient in an inborn error of metabolism ([Chapter 33](#)) [6]. Esterification of carnitine with fatty acyl CoA ester is catalyzed by acyltransferases, such as CPTI ([Chapter 34](#)). The transport of the acylcarnitine across the mitochondrial membrane is

catalyzed by carnitine translocase ([Chapter 35](#)); and then hydrolysis, releasing carnitine and the fatty acylCoA, is catalyzed by a second acyltransferase, CPTII ([Chapters 36 and 37](#)). Inborn errors are known for each of these three enzymatic steps. In β -oxidation, the fatty acid is successively shortened by two carbons, releasing acetyl CoA.

Specific acyl CoA dehydrogenases (ACADs) with

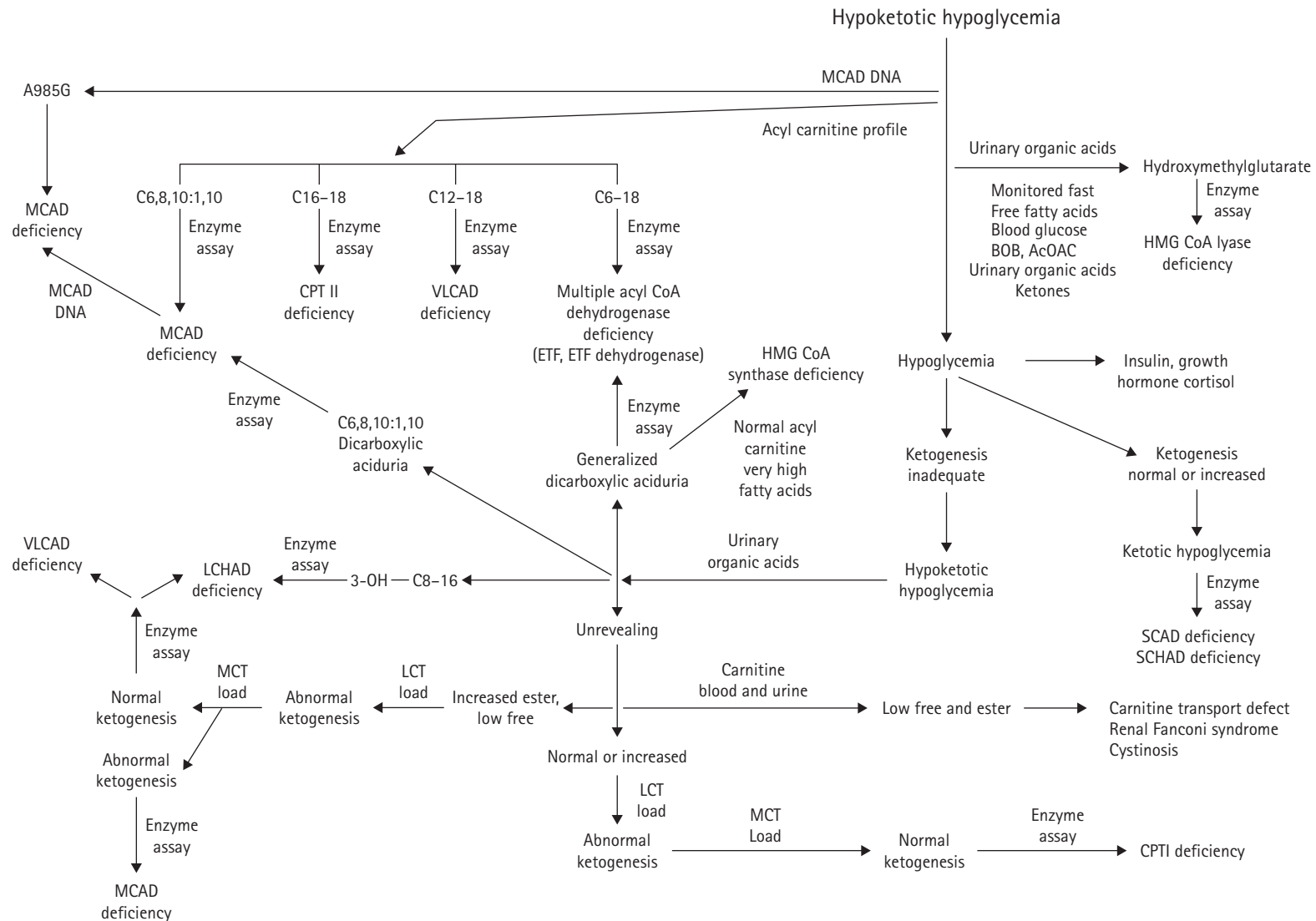


Figure 32.2 Algorithmic approach to the elucidation of hypoketotic hypoglycemia. AcOAc, acetoacetic acid; BOB, 3-hydroxybutyric acid; CPT, carnitine palmitoyltransferase; DNA, deoxyribonucleic acid; ETF, electron transfer flavoprotein; HMG, 3-hydroxy-3-methylglutaric acid; LCHAD, long-chain hydroxyacyl-CoA dehydrogenase; LCT, long-chain triglycerides; MCAD, medium-chain acyl-CoA dehydrogenase; MCT, medium-chain triglycerides; SCAD, short-chain acyl-CoA dehydrogenase; SCHAD, short-chain hydroxyacyl-CoA dehydrogenase; VLCAD, very long-chain acyl-CoA dehydrogenase.

overlapping specificities for chain length include short-chain acyl CoA dehydrogenase (SCAD) (Chapter 41), MCAD (Chapter 38), and very long-chain acyl CoA dehydrogenase (VLCAD) (Chapter 39). In addition, a trifunctional enzyme catalyzes 3-hydroxyacyl dehydrogenation, 2-enoyl-CoA hydration, and 3-oxoacyl CoA thiolysis [7]. Long-chain hydroxyacyl CoA dehydrogenase (LCHAD) is now known to be one of these three enzymatic steps of the trifunctional protein (Chapter 40).

Diseases involving defects in each of these steps have been

defined. Among these diseases, and in five others that have been identified, only MCAD deficiency is easy to diagnose definitively. This is because most MCAD-deficient patients defined prior to expanded newborn screening have had the same mutation, an A→G change at nucleotide 985, which is readily assessed after amplification of the DNA by the polymerase chain reaction. Even in this disease, however, at least five infrequent mutations are known [8], and at least one common mutation T199C found in patients identified by newborn screening. Acylcarnitine profiling should lead

Table 32.1 Acyl carnitine profiles of plasma in the diagnosis of disorders of fatty acid oxidation^a

Disorder	Acylcarnitine	Control reference (μmol/L)	Patient range (μmol/L)	Organic acid analysis
MCAD	C6	0.12	0.12–2.14	Hexanoyl-, suberyl-, phenylpropionyl-, glycine
	C8	0.22	1.28–12.24	
	C8/C8:1	2.32	6.49–46.49	
	C10:1	0.22	0.26–1.84	
LCHAD	C16/C8:1	2.89	10.8–258.96	3-OH-Dicarboxylic, dicarboxylic aciduria
	C16OH	0.02	0.12–0.60	
	C18:10H	0.01	0.14–0.86	
VLCAD	C14:1	0.18	0.76–13.28	Dicarboxylic aciduria
	C14:1/C8:1	1.48	8.26–427.05	
	C14:2	0.08	0.30–3.48	
SCAD/EMA	C4/C3	0.98	0.71–9.0	Ethylmalonic, methylsuccinic aciduria, butyrylglycine
	C4	0.32	0.62–1.28	
	C5/C3	0.80	0.19–4.52	
	C5	0.22	0.16–0.64	
CPTI ^b	C0/C16+18	2–32	63–291	
CPTII	C14:1/C8:1	1.48	2.50–42.79	
	C16/C8:1	2.89	101.24–221.65	
	C16	0.24	2.06–3.94	
	C16:1	0.08	0.50–0.86	
	C18	0.10	0.64–1.411	
	C16+C18/C2	0.011 – 0.095	0.08–0.56	
Multiple AcylCoA-dehydrogenase	C4/C3	0.98	0.70–8.19	Isobutyryl-, isovaleryl-, 2-methyl-buteryl-glycine, glutaric acid, dicarboxylic aciduria
	C5/C3	0.80	1.39–2.01	
	C6	0.12	0.04–0.08	
	C5DC	0.06	0.04–0.08	
Carnitine Transporter	C0	–	↓	
	Cesters	–	↓	
Carnitine Translocase	C16	2.06–3.94	8.85	
	C18	0.64–1.44	↑	
HMG CoA lyase	C5OH	1.06	0.08–1.42	
	Methyl glutaryl	0.02	0.08–0.62	

Adapted from Vreken *et al.* [9] and other sources.

^a Ninety-fifth percentile of the reference range, except where a range is given.

^b Absence of long-chain acyl carnitines.

CPT, carnitine palmitoyl transferase; DC, dicarboxylic acid; EMA, ethylmalonic acidemia.

to the diagnosis in each of these disorders (Table 32.1) [9], but experience is not yet available to indicate how often this approach might miss the diagnosis in an established patient who has become carnitine depleted. Acylcarnitine profiling has also been employed in the analysis of postmortem bile to identify patients with disorders of fatty acid oxidation initially thought to have sudden infant death syndrome [10].

During the long fast, patients must be monitored closely so that symptomatic hypoglycemia is avoided. Testing is best done in units where the staff has experience with the protocol. An intravenous line is placed to ensure access for therapeutic glucose, and bedside monitoring of blood concentrations of glucose is done at regular intervals. In abnormalities of fatty acid oxidation, fasting must be long enough to exhaust stores of glycogen and require the mobilization of fat and its oxidation. This usually requires 17–24 hours.

The specific enzyme assays for specific disorders are technically demanding and not generally available. A good next step following the fast, if a specific disease is not identified, is to pursue a more general study of metabolism in cultured cells [11, 12] in which CoA or carnitine esters are separated and identified by high performance liquid chromatography (HPLC) after interaction with ^{14}C - or ^{13}C -labeled hexadecanoate.

Experience with the diagnosis and management of disorders of fatty acid oxidation has now been summarized for a series of 107 patients with a spectrum of disorders [13]. The severity of these diseases is indicated by the fact that only 57 of the 107 patients were alive at report. An additional 47 siblings had died in infancy for a total of 97 deaths, 30 percent in the first week of life and 69 percent before one year. These data symbolize the importance of newborn screening in prevention, because the avoidance of fasting would prevent many deaths. Seventy-three percent were judged to have hepatic clinical presentations, which included hypoketotic hypoglycemia, hepatomegaly, Reye syndrome, and microscopic hepatic steatosis. True hepatic failure was seen in 11 percent and occurred only in carnitine translocase deficiency and multiple acyl CoA dehydrogenase deficiency; a single patient with LCHAD deficiency had cholestasis. In addition, a previously unrecognized defect in the transport of long chain fatty acids has been reported to cause acute liver failure and hypoketotic hypoglycemia [14]. Oxidation of C14–18 fatty acids by fibroblasts was defective. The clue to the diagnosis was low concentrations of C14–18 fatty acids and elevated carnitine, a very unusual pattern, in biopsied liver. Cardiac presentations were seen in 51 percent of patients. There were arrhythmias, as well as cardiomyopathy. Skeletal muscle involvement in 51 percent of patients included myalgia, myopathy, and rhabdomyolysis with myoglobinuria.

Treatment of disorders of fatty acid oxidation [15] can be considered under two headings: acute management of acute metabolic imbalance and chronic preventive therapy.

Acute management rests on the provision of a plentiful

supply of glucose. This is designed to treat hypoglycemia. It is also designed to inhibit lipolysis. Thus, intravenous solutions of 10 percent glucose or more are the rule even in those who are normal glycemic, for instance a patient with rhabdomyolysis. Insulin, along with glucose, may be necessary to maintain normoglycemia, and a central line or portacath may be required. Carnitine is given preferably intravenously, in doses of 100–300 mg/kg, because of greater bioavailability and the avoidance of diarrhea resulting from large oral doses. Carnitine therapy is mandatory in carnitine transporter deficiency. It has been controversial in long-chain fatty acid disorders because of a theoretical risk that accumulation of long-chain acyl carnitines may be arrhythmogenic, as found in experimental situations [16]. Clinical experience is not consistent with this danger and most support the administration of carnitine, not only to treat the deficiency of free carnitine that develops, but also to promote the detoxifying excretion of accumulated CoA esters as carnitine esters and the restoration of supplies of CoA [17].

The mainstay of long-term management is the avoidance of fasting. Our patients are supplied with letters indicating the need for parenteral glucose whenever intercurrent illness or vomiting preclude the enteral route. We recommend that intravenous glucose be given in this situation even if the blood concentration of glucose is normal on arrival at the emergency room. Overnight fasting is minimized by the use of oral corn starch (1 g/kg hs; 8 g/tbsp). In some situations, continuous nocturnal intragastric feeding has been employed, but we do not recommend it. Restriction of long-chain dietary fat is generally prudent as is long-term oral carnitine. Medium-chain triglyceride supplementation is therapeutic in long-chain fatty acid defects.

REFERENCES

1. Kolvraa S, Gregersen N, Christensen E, Hobolth N. *In vitro* fibroblast studies in a patient with C6-C10-dicarboxylic aciduria: evidence for a defect in general acyl-CoA dehydrogenase. *Clin Chim Acta* 1982; **126**: 53.
2. Stanley CA, Hale DE, Coates PM et al. Medium-chain acyl-CoA dehydrogenase deficiency in children with non-ketotic hypoglycemia and low carnitine levels. *Pediatr Res* 1983; **17**: 877.
3. Naylor EW, Chace DH. Automated tandem mass spectrometry for mass newborn screening for disorders in fatty acid, organic acid, and amino acid metabolism. *J Child Neurol* 1999; **14**(Suppl. 1): S4.
4. Gregersen N, Mortensen PB, Kolvraa S. On the biologic origin of C₆-C₁₀-dicarboxylic and C₆-C₁₀- ω -1-hydroxy monocarboxylic acids in human and rat with acyl-CoA dehydrogenation deficiencies: *in vitro* studies on the ω - and ω -1-oxidation of medium-chain (C6-C12) fatty acids in human and rat liver. *Pediatr Res* 1993; **17**: 828.

5. Marsden D, Nyhan WL, Barshop BA. Creatine kinase and uric acid: early warning for metabolic imbalance resulting from disorders of fatty acid oxidation. *Eur J Pediatr* 2001; **160**: 599.
6. Treem WR, Stanley CA, Finegold DN *et al*. Primary carnitine deficiency due to a failure of carnitine transport in kidney, muscle, and fibroblasts. *N Engl J Med* 1988; **319**: 1331.
7. Carpenter K, Pollitt RJ, Middleton B. Human liver long-chain 3-hydroxyacyl-coenzyme A dehydrogenase is a multifunctional membrane-bound β -oxidation enzyme of mitochondria. *Biochem Biophys Res Commun* 1992; **183**: 443.
8. Yakota I, Coates PM, Hale DE *et al*. Molecular survey of a prevalent mutation, ⁹⁸⁵A-to-G transition, and identification of five infrequent mutations in the medium-chain acyl-CoA dehydrogenase (MCAD) gene in 55 patients with MCAD deficiency. *Am J Hum Genet* 1991; **49**: 1280.
9. Vreken P, van Lint AEM, Bootsma AH *et al*. Rapid diagnosis of organic acidemias and fatty acid oxidation defects by quantitative electrospray tandem-MS acyl-carnitine analysis in plasma. In: Quant PA, Eaton S (eds). *Current Views of Fatty Acid Oxidation and Ketogenesis: From Organelles to Point Mutations*. New York: Plenum Publishers (Kluwer Academic), 1999: 327–37.
10. Rashed MS, Ozand PT, Bennett MJ *et al*. Inborn errors of metabolism diagnosed in sudden death cases by acylcarnitine analysis of postmortem bile. *Clin Chem* 1995; **41**: 1109.
11. Pourfarzam M, Schaefer J, Turnbull DM, Bartlett K. Analysis of fatty acid oxidation intermediates in cultured fibroblasts to detect mitochondrial oxidation disorders. *Clin Chem* 1994; **40**: 2267.
12. Nada M, Rhead W, Sprecher H *et al*. Evidence for intermediate channeling of mitochondrial β -oxidation. *J Biol Chem* 1995; **270**: 530.
13. Saudubray JM, Martin D, De Lonlay P *et al*. Recognition and management of fatty acid oxidation defects: a series of 107 patients. *J Inherit Metab Dis* 1999; **22**: 488.
14. Odaib AA, Shneider BL, Bennett MJ *et al*. A defect in the transport of long-chain fatty acids associated with acute liver failure. *N Engl J Med* 1998; **339**: 1752.
15. Prietsch V, Lindner M, Zschocke J *et al*. Emergency management of inherited metabolic disease. *J Inherit Metab Dis* 2002; **25**: 531–46.
16. Corr PB, Creer MH, Yamada KA *et al*. Prophylaxis of early ventricular fibrillation by inhibition of acylcarnitine accumulation. *J Clin Invest* 1989; **83**: 927.
17. Chalmers RA, Roe CR, Stacey TE, Hoppel CL. Urinary excretion of L-carnitine and acylcarnitines by patients with disorders of organic acid metabolism: evidence of secondary insufficiency of L-carnitine. *Pediatr Res* 1984; **18**: 1325.

Carnitine transporter deficiency

Introduction	253	Treatment	258
Clinical abnormalities	254	References	258
Genetics and pathogenesis	255		

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia, seizures, vomiting, lethargy progressive to coma; cardiomyopathy; chronic muscle weakness; carnitine deficiency in plasma and muscle, and increased excretion of free-carnitine in urine; defective transport of carnitine into cultured fibroblasts and mutations in the *SLC22A5* gene which codes for the sodium ion-dependent carnitine transporter OCTN2.

INTRODUCTION

The inborn errors of fatty acid oxidation, including carnitine transporter deficiency [1, 2], represent a relatively recently recognized area of human disease. The rate of discovery of distinct disorders has increased rapidly since the discovery of medium-chain acyl CoA dehydrogenase (MCAD) deficiency in 1982 (Chapter 38). Deficiency of carnitine is common in these disorders in which fatty acylCoA compounds accumulate which then form esters with carnitine and are preferentially excreted in the urine. Carnitine deficiency may also be profound in organic

acidemias, such as propionic acidemia, for the same reason. The transport of carnitine into fibroblasts is inhibited by long and medium chain acylcarnitines [3], and this may be an additional factor in carnitine deficiency in disorders of fatty acid oxidation. Primary carnitine deficiency resulting from an abnormality in the synthesis of carnitine from protein-bound lysine has not yet been observed. Many of the patients reported early as primary carnitine deficiency have turned out to have MCAD deficiency. Deficiency of carnitine as a result of abnormality in the transporter (Figure 33.1) that facilitates its entry into certain cells has been referred to as primary carnitine deficiency [1]. The

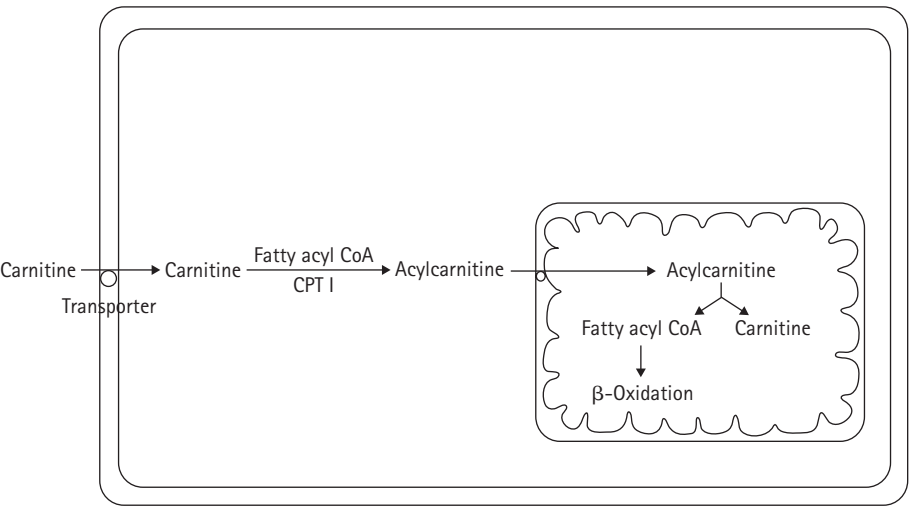


Figure 33.1 The carnitine transporter and its role in fatty acid metabolism.

carnitine transporter is an organic cation transporter (OCTN2) in the solute carrier family. The gene *SLC22A5* has been cloned, and increasing numbers of mutations are being found [4–6].

CLINICAL ABNORMALITIES

The classic, and frequently the initial, presentation of carnitine transporter deficiency (CTD) is hypoketotic hypoglycemia, as in most disorders of fatty acid oxidation. The first patient reported (Figure 33.2) [1] presented at three months, comatose, limp, and unresponsive in the afternoon after a prolonged overnight fast. She was acidotic; the serum bicarbonate was 16 mmol/L and the arterial pH 7.17. The blood concentration of glucose was 0.39 mmol/L (7 mg/dL) and that of the cerebrospinal fluid (CSF) was 0.2 mmol/L (4 mg/dL). Resuscitation required intubation, assisted ventilation, and parenteral glucose and saline. Acute episodes of hypoketotic hypoglycemia are potentially fatal (Figure 33.3) [7] and may be sudden and unexpected. An infant of a vegetarian mother died aged 5 days [8]. Episodes usually occur before two years of age and follow fasting [3, 9].



Figure 33.2 A 17-month-old patient who was the initial reported patient [1] with transporter defect. She presented at three months with hypoglycemic coma precipitated by an intercurrent illness and prolonged fasting. This episode left her with severe brain damage reflected in her vacant expression. She died shortly after the picture was taken because of complications of a gastrostomy. (Illustration was kindly provided by Dr Charles Stanley of the Children's Hospital of Philadelphia.)

Modest hepatomegaly is characteristic of this condition. Biopsy of the liver shows microvesicular lipid [10], a finding, like the rest of this clinical picture, that might lead to a diagnosis of Reye syndrome.

Clinical chemistry in the acute hypoketotic episode is also consistent with Reye syndrome, with hyperammonemia and increased levels of transaminases. The initial patient had an ammonia of 338 mmol/L, slightly prolonged prothrombin time, an aspartate transaminase (AST) of 248 and alanine transaminase (ALT) of 149 IU/L [1]. Recurrent episodes of Reye syndrome have been reported. Uric acid concentrations are also elevated at the time of the episode. This, along with the elevation of creatine phosphokinase (CK) [3] should strongly suggest a disorder of fatty acid oxidation.

Examination of the urine may reveal no ketonuria [1, 3], and this should strongly suggest the diagnosis. However, in the height of the hypoglycemic illness, there may be misleading ketonuria in any disorder of fatty acid oxidation. Quantification of the plasma concentration of 3-hydroxybutyric acid or of acetoacetic and 3-hydroxybutyric acids at this time will provide definitive evidence of impaired ketogenesis, but this information is not usually available to the clinician. Dicarboxylic aciduria is usually notably absent [1, 7].

Cardiomyopathy is the other classic way in which this disorder presents [3, 7, 10] and may be expected in any patient not given the benefit of diagnosis and treatment with carnitine. It was the most common presenting complaint in 15 patients [6] and present in 100 percent of 20 early reported patients. The patient of Waber and colleagues [10] reported with progressive cardiomyopathy and cardiac failure successfully treated with carnitine was subsequently shown to have the transporter defect. A median age of onset of cardiac symptoms was three years [3]. Cardiomyopathy and congestive cardiac failure has begun at seven years of age. Onset may be with rapidly progressive heart failure [3] or a murmur, and cardiomegaly may be found on routine physical examination or examination at the time of hypoglycemia. Roentgenograms and echocardiography reveal cardiac enlargement and increased thickness of the left ventricular wall. Electrocardiogram (EKG) reveals left ventricular hypertrophy. Nevertheless, the cardiomyopathy has also been described as characteristically dilated [11], and this has repeatedly been confirmed by cardiac catheterization. This may be expected to be a lethal disease in which patients without carnitine supplementation display cardiac failure progressive to death. Cardiac disease may also present with arrhythmia. Death in a sibling has been recorded in at least eight families [3]. A 12-year-old boy who died suddenly of cardiomyopathy following a routine surgical procedure was found to have a low concentration of carnitine in plasma and defective transport of carnitine in fibroblasts [12].

Muscle weakness or hypotonia is the third major manifestation of disease. It may be present along with

other features, particularly those of the heart, but in two patients it was the only manifestation [3]. The picture may be that of a progressive proximal myopathy [11]. Biopsy of muscle reveals lipid storage myopathy [1].

An unusual presentation was that of an infant with profound peripheral neuropathy [13]; she had absent deep tendon reflexes and could not walk. Electromyogram (EMG) was consistent with sensory and motor neuropathy, and muscle biopsy reported neurogenic atrophy. Delay in diagnosis has been another characteristic of this disease. In nine patients, the delay was between one and six years after the onset of symptoms [3], and in this time all developed cardiomyopathy, and all but one had muscle weakness. In some patients, mild muscle weakness may not have been noted because of the attention devoted to the major cardiac manifestations.

The advent of newborn screening for this disease made it clear that it can also be asymptomatic well into adult life [14–16]. In programs of newborn screening using tandem mass spectrometry (MS/MS), it has become a common occurrence to find that a positive newborn screen for C_0 -deficiency results from an asymptomatic but affected mother. Confronted with an abnormal C_0 -screen, we all now study the mother as well as the baby. In 15 reported families in which the defective maternal transporter was confirmed by studies of uptake of carnitine by fibroblasts, mutational analysis, or both, the infant was normal in all but one; in that family, mother and infant were affected. Most mothers were asymptomatic. Three had low stamina, easy fatigability with exercise and fasting intolerance [16]. Echocardiograms in four were normal [14].

Asymptomatic status has been observed in a father and son with defective fibroblast uptake of carnitine and the same mutations as another who presented in infancy with a Reye-like syndrome of hepatic disease and encephalopathy [17].

Tandem mass spectrometry has made screening for this disease an integral part of most programs of newborn screening [18].

GENETICS AND PATHOGENESIS

Transmission of the disorder is autosomal recessive. Affected siblings of both sexes have been observed, and consanguinity has been present in at least five families [3, 19]. Prevalence is not yet known, but there were ten patients in the series of 107 with disorders of fatty acid oxidation in the experience of Saudubray *et al.* [11] in Paris, and newborn screening experience should soon give reliable prevalence data. Among 313 patients with an autopsy diagnosis of sudden infant death syndrome (SIDS), three were designated as transporter defects on the basis of hepatic steatosis and very low hepatic carnitine along with low esterified carnitine [20].

Prior to newborn screening, the diagnosis was usually suspected on the basis of a low concentration of free-carnitine in plasma. In the first patient, the total plasma carnitine ranged from 0 to 2.2 mmol/L and no free-carnitine could be detected. In 20 patients [3], total plasma carnitine ranged from 0 to 9 mmol/L, with 18 having values less than 4.2 mmol/L. In controls, total carnitine was 40–60 mmol/L (Table 33.1). The acylcarnitine profile reveals a decrease in free and esterified carnitines.

Concentrations of carnitine in muscle are also quite low. In 13 patients studied, the range for total carnitine was from 0.05 to 17 percent of the normal mean. In liver, the total was 5 percent of normal. The excretion of carnitine in the urine is inappropriately high, consistent with defective renal tubular reabsorption [10]. At a time when the plasma carnitine approximated zero, the renal excretion was 126 mmol/g creatinine (normal, 167–425) [1], and following a dose of 100 mg/kg of oral carnitine, the plasma carnitine rose only to 21 mmol/L, but urinary excretion increased to 2911 mmol/g creatinine. After four months of carnitine treatment, the plasma concentrations reached the low normal range, while urinary excretion was four to five times normal. The fractional excretion rate for free-carnitine was nearly 100 percent of the filtered load. On withdrawal of treatment, the fractional excretion exceeded the filtered load.

Table 33.1 Differential diagnosis of disorders involving carnitine

	Plasma total (mmol/L)	Carnitine esterified (% of total)	Urinary carnitine
Control	40–60	30	Normal
Carnitine transporter deficiency	5	30	Paradoxically high free
Carnitine palmitoyl transferase (CPT) I deficiency	60–100	20	Normal or high
Carnitine translocase deficiency	5–30	80–100	High ester
Carnitine palmitoyl transferase (CPT) II deficiency	10–40	40–80	Normal or high ester
Defects in β -oxidation	10–30	30–60	High ester
3-Hydroxy-3-methylglutaryl CoA lyase deficiency	10–30	30–60	High ester



Figure 33.3 JS: A 12-year-old boy with carnitine transporter deficiency. The disease is exquisitely responsive to carnitine. His death at 13 years highlights the dangerous nature of the disease and the importance of close follow up of carnitine status and expert management.

The nature of the defect has been demonstrated by study of the uptake of carnitine *in vitro* by cultured fibroblasts (Figure 33.3) [1, 3]. In control cells, the uptake of ^{14}C -labeled carnitine was via a high-affinity, carrier-mediated transport process with an apparent K_m of 3.24 ± 0.5 and a V_{\max} of 1.67 ± 0.19 [1] consistent with previous reports [21]. Fibroblasts from patients have shown little uptake of carnitine; at a concentration of carnitine of 5 mmol/L, control uptake was 0.94 and a patient uptake was 0.1 pmol/min per mg protein [1]. High-affinity transport is best shown at lower concentrations; up to 1 mmol/L uptake was negligible. Uptake in patients at high concentrations, such as 10 or 20 mmol/L reflect a second low-affinity transporter [20] or passive diffusion [22]. Transport of carnitine in control fibroblasts is sodium-dependent [1]. The uptake of carnitine by fibroblasts at 5 mmol/L showed no overlap among patients and controls. The velocity of carnitine uptake can be measured in lymphoblasts, as well as fibroblasts [23]. Patients display rates below 10 percent of control. Heterozygosity can be demonstrated in some patients by rates below 40 percent of control. Low uptake of carnitine has also been demonstrated in cultured myocytes derived from patients [24]. Prenatal diagnosis has been accomplished by demonstration of defective uptake of carnitine from amniocytes of an affected fetus [25].

In response to the administration of carnitine, levels in liver return to normal, while those in muscle respond poorly, indicating that the transport defect includes muscle

and kidney, but not liver. Consistent with this, the low K_m and preference for L-isomer that characterize the uptake of carnitine by fibroblasts is shared by heart and muscle [21, 22, 26], but not by liver [27].

The gene for the carnitine transporter, *SLC22A5*, has been mapped to chromosome 5q31, the locus for carnitine transporter deficiency in a large Japanese kindred [26]. The gene has ten exons over 26 kb. It codes for the organic cation transporter OCTN2, which is one of a family of organic cation (OCTN) sodium ion-dependent transporters [28, 29]. The protein contains 557 amino acids and has the properties of a high-affinity transporter. Many mutations have now been identified in patients with this disease [3, 30–38]. Most individual families have had unique mutations. There is an OCTN2 database (www.arup.utah.edu/database/OCTN/OCTN2). There have been a few instances of the same mutation in unrelated patients [5, 32–36]. A few stop codons and frame shifts have been defined [3, 30]. A lack of correlation between genotype and phenotype has been discussed [3, 36]. However, decisions as to severity of phenotype often rest on whether or not hypoglycemia once occurred early in life. Differences in presentation could simply reflect the chance occurrence of an intercurrent illness that led to fasting.

Among ethnic differences, an 11-bp deletion was found in unrelated patients from Switzerland and neighboring northern Italy [3] and R169W was found in two unrelated families in Italy [36]. In Japan, where the disease appears relatively frequent, most families have had a few mutations [39]. In a survey of 973 unrelated Japanese [39], 14 were found to have low levels of carnitine, and of these, six had mutations in the gene for OCTN2: W132X, S467C, W283C, and M179L. These data gave a carrier frequency of 1 percent in Japan. Echocardiographic study indicated asymptomatic cardiac hypertrophy in these heterozygotes. Two Iranian Jewish siblings with the same mutation (R399Q) had very different clinical presentations [40]. One presented in coma at two years of age following gastroenteritis. Her older sibling had proximal limb girdle weakness, which was markedly improved following treatment with carnitine. In studies with confocal microscopy, some mutant OCTN2 matured normally to the plasma membrane, while others were retained in the cytoplasm [41].

An animal model of the carnitine transporter defect, the juvenile visceral steatosis (jvs) mouse [42], has autosomal recessive fatty infiltration of the liver, hypoglycemia, and hyperammonemia 2 weeks after birth, and very low levels of carnitine in blood and muscle, along with defective renal reabsorption of free carnitine. The hyperammonemia results from decreased expression of genes for enzymes of the urea cycle; low levels of mRNA are associated with low levels of all of the hepatic enzymes of the urea cycle [43]. Treatment with carnitine corrects the abnormal expression and urea cycle enzyme activity [44]. The jvs gene has been mapped to mouse chromosome 11, which is syntenic with the *SLC22A5* locus on human chromosome 5 [45].

Analysis of the organic acids of the urine of these patients

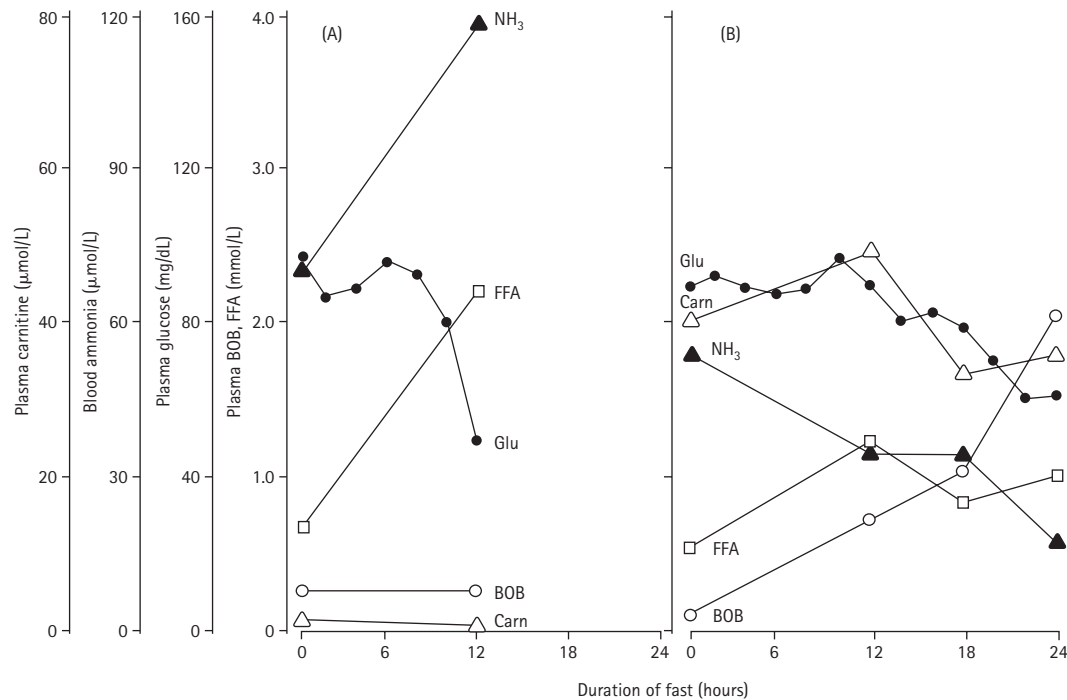


Figure 33.4 The response to fasting in a patient with the carnitine transporter defect. (A) In the control state, hypoglycemia (glu) was prominent at 12 hours, and there was no evident ketogenesis (3-hydroxybutyrate (BOB)) despite elevation of free-fatty acids (FFA). (B) Following treatment with carnitine, fasting for 24 hours was without hypoglycemia, and ketogenesis was evident in the rising BOB. (Reprinted with permission from the *New England Journal of Medicine* [1].)

is usually normal. The absence of dicarboxylic aciduria, especially at times of acute illness and hypoglycemia, contrasts sharply with findings in patients with defects in β -oxidation, such as MCAD deficiency. Patients with deficiency of carnitine palmitoyl-transferase I (Chapter 35) also develop hypoketotic hypoglycemia without dicarboxylic aciduria [46]. Comparisons of alterations of plasma carnitine in various disorders are shown in Table 33.1. Low free and total carnitine in plasma along with urinary free carnitine that is paradoxically maintained is suggestive of a transporter defect.

The response to fasting in a patient with defective carnitine transporter showed hypoketosis throughout and hypoglycemia by 12 hours (Figure 33.4) [1]. The fast was stopped when the plasma glucose reached 2.8 mmol/L (51 mg/dL), at which time the patient remained asymptomatic. Levels of free-fatty acids in plasma rose sharply to 2.22 mmol/L, but the level of 3-hydroxybutyrate remained flat at 0.27 mmol/L. Blood concentrations of ammonia rose. Treatment with carnitine corrected this patient's impaired hepatic oxidation of fatty acids; and she was able to fast for 24 hours without hypoglycemia. Levels of 3-hydroxybutyrate rose to 2 mmol/L, higher than the free-fatty acids (1.25 mmol/L).

Diet may contribute to the pathogenesis of symptoms in this disease. A 12-year-old patient who died suddenly following surgery [13] had been exposed to an essentially vegetarian diet for some time. The three-month-old initial

patient [1] had been changed from a cow's milk protein containing formulation to a soy protein preparation that contained no carnitine, 4 weeks prior to the episode of hypoketotic hypoglycemia.

The pathogenesis of symptoms of hypoketotic hypoglycemia reflects the role of fat in energy metabolism. Hypoglycemia after short periods of fasting usually represent disorders of carbohydrate metabolism. The oxidation of fatty acids is not a major source of energy until relatively late in fasting. It usually takes 15–24 hours of fasting to induce hypoglycemia in a patient with a disorder of fatty acid oxidation. An individual who never fasted beyond 12 hours would usually be protected against this manifestation.

The metabolism of fat begins with lipolysis; those patients with defective fatty acid oxidation have high ratios of free fatty acids to 3-hydroxybutyrate in blood after fasting. Once transported into cells carnitine is esterified with acyl CoA esters, including those of fatty acids resulting from lipolysis. The esterifications are catalyzed by carnitine acyl transferases, such as carnitine palmitoyl transferase (CPT I). Carnitine translocase then catalyzes the transfer of the fatty acylcarnitines across the membrane into the mitochondrion, where hydrolysis to fatty acyl CoA and free or recycled carnitine is catalyzed by CPT II. Fatty acyl CoA compounds then undergo β -oxidation in which there is successive shortening by two carbon atoms releasing acetyl CoA. In muscle, this is largely oxidized via the citric acid

cycle, while in liver ketogenesis proceeds via the successive action of 3-hydroxymethylglutaryl (HMG) CoA synthase and lyase-yielding acetoacetate, which is converted to 3-hydroxybutyrate.

TREATMENT

Treatment of this disease with carnitine has been highly successful [3, 10]. Levels of free-carnitine in plasma and liver are readily restored, preventing further attacks of hypoketotic hypoglycemia. Developmental delay or seizures induced by hypoglycemic attacks prior to treatment persist, but are not progressive.

Cardiomyopathy and failure respond dramatically to treatment [9, 10]. Heart size is reduced to normal within months. Doses have ranged from 50 to 120 mg/kg p.o. Doses as high as 400 mg/kg have been recommended [15]. Intestinal tolerance often mandates lower dosage. Skeletal muscle weakness improved, although mild proximal muscle weakness has occasionally persisted. However, muscle concentrations of carnitine were documented to increase only slightly to 22–80 mmol/g; control levels are 2500–3500 mmol/g. These observations suggested that muscle oxidation of fat and muscle function may be unaffected until the intracellular muscle concentration of carnitine falls below 30–50 mmol/L or 2–4 percent of normal. Biopsied muscle revealed a decrease of stored lipid with treatment, but not a disappearance [1].

The occurrence of asymptomatic mothers uncovered by programs of newborn screening raises questions as to treatment. It appears prudent to treat these women [15]. This may be particularly true during a subsequent pregnancy, as pregnancy lowers carnitine stores.

Studies which indicated failure of mutant transporter proteins to mature normally to the plasma membrane [41] led to studies of the effects of small molecules on carnitine transport *in vitro*. Phenylbutyrate, quinidine, and verapamil were found to stimulate transport raising the possibility of pharmacologic therapy.

REFERENCES

1. Treem WR, Stanley CA, Finegold DN *et al*. Primary carnitine deficiency due to a failure of carnitine transport in kidney muscle and fibroblasts. *N Engl J Med* 1988; **319**: 1331.
2. Lindstedt S, Eriksson BO, Nordin I. Hereditary defect in carnitine membrane transport is expressed in skin fibroblasts. *Eur J Pediatr* 1988; **147**: 662.
3. Stanley CA, DeLeeuw S, Coates PM *et al*. Chronic cardiomyopathy and weakness of acute coma in children with a defect in carnitine uptake. *Ann Neurol* 1991; **30**: 709.
4. Nezu J, Tamai I, Oku A *et al*. Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter. *Nat Genet* 1999; **21**: 91.
5. Wang Y, Ye J, Ganapathy V, Longo N. Mutations in the organic cation/carnitine transporter OCTN2 in primary carnitine deficiency. *Proc Natl Acad Sci USA* 1999; **96**: 2356.
6. Wang Y, Horman SH, Ye J *et al*. Phenotype and genotype variation in primary carnitine deficiency. *Genet Med* 2001; **3**: 387.
7. Brivet M, Boutron A, Slama A *et al*. Defects in activation and transport of fatty acids. *J Inherit Metab Dis* 1999; **22**: 428.
8. Rinaldo P, Stanley CA, Hsu B *et al*. Sudden neonatal death in carnitine transporter deficiency. *J Pediatr* 1997; **131**: 304.
9. Tein I, De Vivo DC, Bierman F *et al*. Impaired skin fibroblast carnitine uptake in primary systemic carnitine deficiency manifested by childhood carnitine-responsive cardiomyopathy. *Pediatr Res* 1990; **28**: 217.
10. Waber LJ, Valle D, Neill C *et al*. Carnitine deficiency presenting as familial cardiomyopathy: a treatable defect in carnitine transport. *J Pediatr* 1982; **101**: 700.
11. Saudubray JM, Martin D, De Lonlay P *et al*. Recognition and management of fatty acid oxidation defects: a series of 107 patients. *Arch Neurol* 2004; **61**: 570.
12. Pollitt RJ, Olpin SE, Bonham JR *et al*. Late-presenting carnitine transport defect. *Enzyme Prot* 1993; **47**: 175.
13. Makhseed N, Vallance HD, Potter M *et al*. Carnitine transporter defect due to a novel mutation in the SLC22A5 gene presenting with peripheral neuropathy. *J Inherit Metab Dis* 2004; **27**: 778.
14. El-Hattab AW, Li FY, Shen J *et al*. Maternal systemic primary carnitine deficiency uncovered by newborn screening: clinical, biochemical, and molecular aspects. *Genet Med* 2010; **12**: 19.
15. Vijay S, Patterson A, Olpin S *et al*. Carnitine transporter defect: diagnosis in asymptomatic adult women following analysis of acylcarnitines in their newborn infants. *J Inherit Metab Dis* 2006; **29**: 627.
16. Schimmenti LA, Crombez EA, Schwahn BC. Expanded newborn screening identifies maternal primary carnitine deficiency. *Mol Genet Metab* 2007; **90**: 441.
17. Spiekerkoetter U, Huener G, Baykal T *et al*. Silent and symptomatic primary carnitine deficiency within the the same family due to identical mutations in the organic cation carnitine transporter OCTN2. *J Inherit Metab Dis* 2003; **26**: 613.
18. Wilcken B, Wiley V, Giak Sim K *et al*. Carnitine transporter defect diagnosed by newborn screening with electrospray tandem mass spectrometry. *J Pediatr* 2001; **138**: 581.
19. Rahbeeni Z, Vaz FM, Al-Hussein K *et al*. Identification of two novel mutations in OCTN2 from two Saudi patients with systemic carnitine deficiency. *J Inherit Metab Dis* 2002; **25**: 363.
20. Boles RG, Buck EA, Blitzer MG *et al*. Retrospective biochemical screening of fatty acid oxidation disorders in postmortem livers of 418 cases of sudden death in the first year of life. *J Pediatr* 1998; **132**: 924.
21. Rebouche CJ, Engel AG. Carnitine transport in cultured muscle cells and skin fibroblasts from patients with primary systemic carnitine deficiency. *In Vitro* 1982; **18**: 495.
22. Vary TC, Nealy JR. Characterization of carnitine transport in isolated perfused adult rat hearts. *Am J Physiol* 1982; **242**: H585.

23. Tein I, Xie ZQ. The human plasmalemmal carnitine transporter defect is expressed in cultured lymphoblasts: a new non-invasive method for diagnosis. *Clin Chim Acta* 1996; **252**: 201.
24. Pons R, Carrozzo R, Tein I *et al*. Deficient muscle carnitine transport in primary carnitine deficiency. *Pediatr Res* 1997; **42**: 583.
25. Christodoulou J, Teo SH, Hammond J *et al*. First prenatal diagnosis of the carnitine transporter defect. *Am J Med Genet* 1996; **66**: 21.
26. Rebouche CJ. Carnitine movement across muscle cell membranes: Studies in isolated rat muscle. *Biochim Biophys Acta* 1977; **471**: 145.
27. Christiansen RZ, Bremer J. Active transport of butyrobetaine and carnitine into isolated liver cells. *Biochim Biophys Acta* 1976; **448**: 562.
28. Wu X, Prasad PD, Leibach FH, Ganapathy V. cDNA sequence transport function and genomic organization of human OCTN2 a new member of the organic cation transporter family. *Biochem Biophys Res Commun* 1998; **246**: 589.
29. Tamai I, Ohashi R, Nezu J *et al*. Molecular and functional identification of sodium ion-dependent high affinity human carnitine transporter OCTN2. *J Biol Chem* 1998; **273**: 20378.
30. Lamhonwah AH, Tein I. Carnitine uptake defect: frameshift mutations in the human plasmalemmal carnitine transporter gene. *Biochem Biophys Res Commun* 1998; **252**: 396.
31. Tang NL, Ganapathy V, Wu X *et al*. Mutations of OCTN2 an organic cation/carnitine transporter lead to deficient cellular carnitine uptake in primary carnitine deficiency. *Hum Mol Genet* 1999; **8**: 655.
32. Burwinkel B, Kreuder J, Schweitzer S *et al*. Carnitine transporter OCTN2 mutations in systemic primary carnitine deficiency: a novel Arg169Gln mutation and a recurrent Arg282Ter mutation associated with an unconventional splicing abnormality. *Biochem Biophys Res Commun* 1999; **161**: 484.
33. Vaz FM, Scholte HR, Ruiters J *et al*. Identification of two novel mutations in OCTN2 of three patients with systemic carnitine deficiency. *Hum Genet* 1999; **105**: 157.
34. Mayatepek E, Nezu J, Tamai I *et al*. Two novel missense mutations of the OCTN2 gene (W282R and V446F) in a patient with primary systemic carnitine deficiency. *Hum Mutat* 2000; **15**: 118.
35. Wang Y, Kelly MA, Cowan TM, Longo N. A missense mutation in the OCTN2 gene associated with residual carnitine transport activity. *Hum Mutat* 2000; **15**: 238.
36. Wang Y, Taroni F, Garavaglia B, Longo N. Functional analysis of mutations in the OCTN2 transporter causing primary carnitine deficiency: lack of genotype-phenotype correlation. *Hum Mutat* 2000; **16**: 401.
37. Cederbaum S, Dipple K, Vilain E *et al*. Clinical follow-up and molecular etiology of the original case of carnitine transporter deficiency. *J Inher Metab Dis* 2000; **23**(Suppl. 1): 119.
38. Christensen E, Holm J, Hansen SH *et al*. Sudden infant death following pivampicillin treatment in a patient with carnitine transporter deficiency. *J Inher Metab Dis* 2000; **23**(Suppl. 1): 117.
39. Koizumi A, Nozaki J, Ohura T *et al*. Genetic epidemiology of the carnitine transporter OCTN2 gene in a Japanese population and phenotypic characterization in Japanese pedigrees with primary systemic carnitine deficiency. *Hum Mol Genet* 1999; **8**: 2247.
40. Wang Y, Korman SH, Ye J *et al*. Phenotype and genotype variation in primary carnitine deficiency. *Genet Med* 2001; **3**: 387.
41. Di San Filippo CA, Pasquali M, Longo N. Pharmacological rescue of carnitine transport in primary carnitine deficiency. *Hum Mutat* 2006; **27**: 513.
42. Horiuchi M, Hayakawa J, Yamaguchi S, Saheki T. Possible primary defect of juvenile visceral steatosis (jvs) mouse with systemic carnitine deficiency. First IUBMB Conference, Biochemistry of Diseases. Nagoya Congress Center, 1992: (Abstr. 2-a-05-p8).
43. Tomomura M, Yasushi I, Horiuchi M *et al*. Abnormal expression of urea cycle enzyme genes in juvenile visceral steatosis (jvs) mice. *Biochim Biophys Acta* 1992; **1138**: 167.
44. Horiuchi M, Kobayashi K, Tomomura M *et al*. Carnitine administration to juvenile visceral steatosis mice corrects the suppressed expression of urea cycle enzyme by normalizing their transcription. *J Biol Chem* 1992; **267**: 5032.
45. Nikaido H, Horiuchi M, Hashimoto N *et al*. Mapping of jvs (juvenile visceral steatosis) gene which causes systemic carnitine deficiency in mice on chromosome 11. *Mamm Genome* 1995; **6**: 369.
46. Bonnefont JP, Haas R, Wolff J *et al*. Deficiency of carnitine palmitoyltransferase I. *J Child Neurol* 1989; **4**: 197.

Carnitine-acylcarnitine translocase deficiency

Introduction	260	Treatment	265
Clinical abnormalities	261	References	265
Genetics and pathogenesis	264		

MAJOR PHENOTYPIC EXPRESSION

Episodes of life-threatening illness with cardiac arrhythmia; coma with hypoketotic hypoglycemia, and hyperammonemia; sudden infant death; hepatomegaly; muscle weakness; deficiency of free carnitine and increased long-chain acylcarnitines; and deficiency of carnitine translocase.

INTRODUCTION

Carnitine translocase (carnitine:acylcarnitine carrier) deficiency is a recently discovered disorder of fatty acid oxidation. First described in 1992 [1], the disease accounted for ten of 107 patients in the Saudubray experience with abnormalities in the oxidation of fatty acids [2]. Many patients have developed symptoms and died in infancy [1–4].

Mitochondrial oxidation of long-chain fatty acids provides an important source of energy for the heart, as well as for skeletal muscle during prolonged aerobic work and for hepatic ketogenesis during long-term fasting. The carnitine shuttle is responsible for transferring long-chain fatty acids across the barrier of the inner mitochondrial membrane to gain access to the enzymes of β -oxidation. The shuttle consists of three enzymes (carnitine

palmitoyltransferase I, carnitine-acylcarnitine translocase (CACT), carnitine palmitoyltransferase 2) and a small, soluble molecule (carnitine) to transport fatty acids as their long-chain fatty acylcarnitine esters. Carnitine is provided in the diet (animal protein) and also synthesized at low rates from trimethyllysine residues generated during protein catabolism. Carnitine turnover rates (300–500 $\mu\text{mol/day}$) represent <1 percent of body stores; 98 percent of carnitine stores are intracellular (total carnitine levels are 40–50 μM in plasma versus 2–3 mM in tissue). Carnitine is removed by urinary excretion after reabsorption of 98 percent of the filtered load; the renal carnitine threshold determines plasma concentrations and total body carnitine stores [5].

Long chain fatty acids must be esterified with carnitine before they can be transported into the mitochondria where β -oxidation takes place. The translocase, carnitine-acylcarnitine translocase, catalyzes the transfer of the

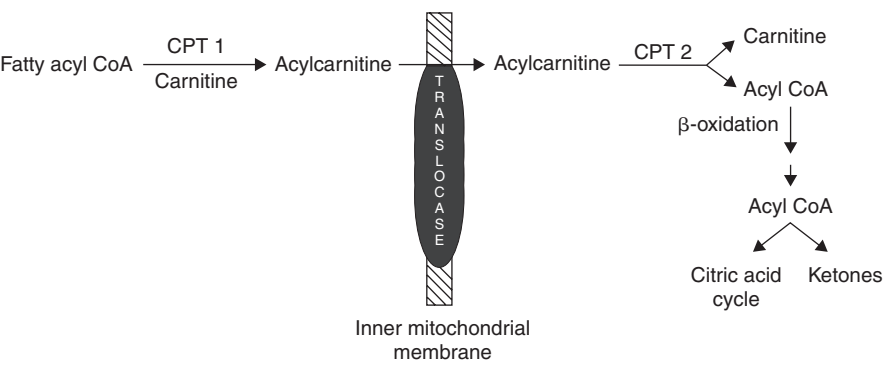


Figure 34.1 Long chain fatty acid oxidation and the role of carnitine translocase. CPT, carnitine palmitoyltransferase.

acylcarnitines across the inner mitochondrial membrane (Figure 34.1). The enzyme is one of ten related membrane carrier proteins that shuttle proteins from the cytosol to the mitochondrial matrix. Another is the ornithine transporter that is defective in the HHH (hyperornithinemia, hyperammonemia, homocitrullinuria) syndrome (Chapter 30). Once inside the mitochondrion, the acylcarnitine is split through the action of carnitine palmitoyltransferase (CPT) II to free carnitine and the fatty acyl CoA ester, which is then the substrate for β -oxidation. Each step in the sequence is essential if fat is to be burned as fuel or converted to ketones and used for gluconeogenesis. Carnitine:acylcarnitine translocase and early-onset CPTII (Chapter 36) deficiencies have an extremely high neonatal mortality rate. Late-onset CPTII deficiency (Chapter 37) is characterized only by episodic rhabdomyolysis. CPT type IA deficiency (Chapter 35) may often be benign, although early presentation with hypoketotic hypoglycemia certainly occurs [6].

The gene has been cloned and some mutations identified [7].

CLINICAL ABNORMALITIES

The hallmark of disorders of fatty acid oxidation is hypoketotic hypoglycemia (Figure 34.2), and this has often occurred in the neonatal period in this disease [4]. Hyperammonemia, encephalopathy, cardiomyopathy, hepatopathy, and myopathy are typically seen in the neonatal period [8, 9]. The major characteristic of this disorder has been the occurrence of cardiac arrhythmias [1–4, 9].

Episodes typically follow prolonged fasting, which is a common response of infants to intercurrent infectious disease. In one patient [3], a dextrostix reading of zero was recorded during an episode at 36 hours in which the infant was found to be pale, unresponsive, and hypothermic (34.5°C). Another patient [1] had a seizure, apnea, and bradycardia at 36 hours of age. This episode, which appeared to have been provoked by fasting, led to apnea requiring mechanical ventilation and hypotension, which was treated with lidocaine and dopamine. This patient went on to have repeated episodes of vomiting, lethargy, and coma following intercurrent illness and attendant fasting; each responded to the intravenous administration of glucose. Another patient [3] developed a second episode of hypoglycemia (0.7 mmol/L) on the third day of life, despite receiving 5 percent glucose intravenously; the test for ketones in the urine was negative. The glucose was corrected by increasing the rate of infusion of glucose, but the patient deteriorated clinically and died at 8 days of age. Undetectable glucose was also the case in a patient who presented at 36 hours [10].

A previous sibling of the first patient [1] died at 4 days of age of what might be interpreted as sudden infant death syndrome. He had a sudden, unexplained cardiorespiratory

arrest at 2 days and died 2 days later. The previous sibling of another patient [10] died of cardiorespiratory arrest at 24 hours. Two previous siblings of a patient with the disease, who died suddenly at 12 months, had died in the first 12 months [11].

Cardiomyopathy may be manifested by premature ventricular contractions, ventricular tachycardia, or hypotension [1], and bradycardia due to auriculoventricular block [3]. In one patient [1], the electrocardiogram showed ventricular hypertrophy and in another [3], a left bundle branch block. Echocardiogram showed reduced ejection fraction. Intracardiac conduction defects were seen in twin siblings who died after an episode days after onset at two months [12]. Over a period of 25 years, in 107 newborns, cardiac arrhythmia and conduction defects were the main presentations in patients with fatty acid oxidation defects. Conduction disorders and atrial tachycardias were observed in patients with defects of long-chain fatty acid transport across the inner mitochondrial membrane (carnitine palmitoyl transferase type II deficiency (Chapter 36) and carnitine-acylcarnitine translocase deficiency) and in patients with trifunctional protein deficiency (Chapter 40). Arrhythmias have been attributed to the accumulation of intermediary metabolites of fatty acids, such as long-chain acylcarnitines [13].

Muscle disease may be seen very early in this disorder [4]. Weakness and hypotonia may be manifest first in poor head control, and later inability to walk further than 15



Figure 34.2 One-month-old Saudi female with carnitine-acylcarnitine translocase deficiency. She presented with severe hypoketotic hypoglycemia, hyperammonemia, and coma. She also had a cardiomyopathy that improved. She required peritoneal dialysis and ventilation. She was discharged home at three months of age in good condition [8]. A unique molecular defect (p.Q238R) was found; the family underwent preimplantation genetic diagnosis and had a normal child [7].

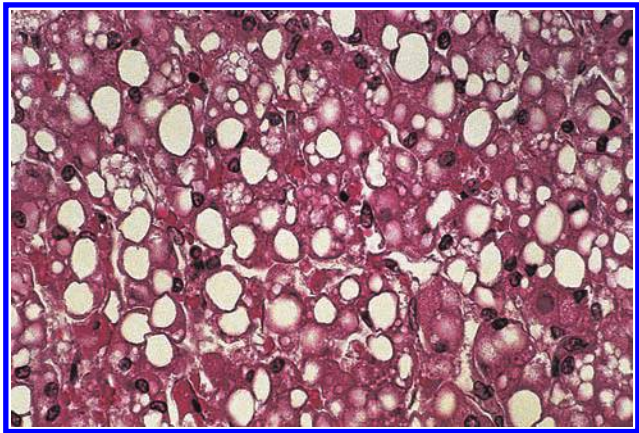


Figure 34.3 Biopsied liver of a patient with carnitine translocase deficiency. This H&E-stained section reveals extensive deposition of fat. (This illustration was kindly provided by Dr Jean-Marie Saudubray of l'Hopital des Enfants Malades, Paris.)

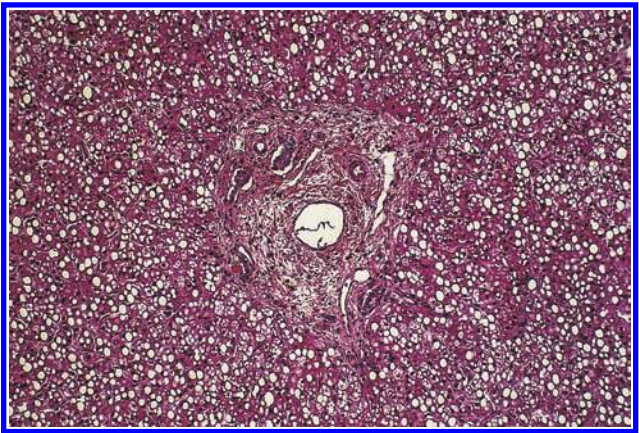


Figure 34.4 Biopsied liver. The fat followed both microvesicular and macrovesicular patterns of steatosis. (This illustration was kindly provided by Dr Jean-Marie Saudubray of l'Hopital des Enfants Malades, Paris.)

feet [1]. The level of creatine kinase (CK) in the blood may be elevated [4, 10, 14]. Hypertonia may develop during terminal coma [1].

Hepatomegaly is a regular occurrence in this disease, and size tends to increase with time. Hepatic failure has also been recorded [2, 14]. In one patient, there was nephromegaly. Histological examination of the liver may reveal massive macrovesicular steatosis (Figures 34.3 and 34.4) [2, 3], as well as some fibrosis. Muscle histology has been normal. Mental development and growth has been normal [1], but most of these patients have died in infancy. One patient [10] developed microcephaly. Terminal episodes in most were cardiorespiratory failure and cardiac

arrhythmia. In one, there was a pulmonary hemorrhage and death at 8 days of life [3].

In addition to the hypoglycemia and deficient ketogenesis, clinical laboratory data have included hyperammonemia (491, 270, and 272 $\mu\text{mol/L}$) [1, 3, 14]. Patients were treated with sodium benzoate [1, 14]. In some patients, a urea cycle defect was considered. Some have had hyperammonemia in infancy without hypoglycemia or any of the other manifestations of a deficit in fatty acid oxidation [14]. Orotic acid excretion is, however, normal [9].

During the acute episode, creatine phosphokinase levels in the blood as high as 4595 IU/L have been recorded,

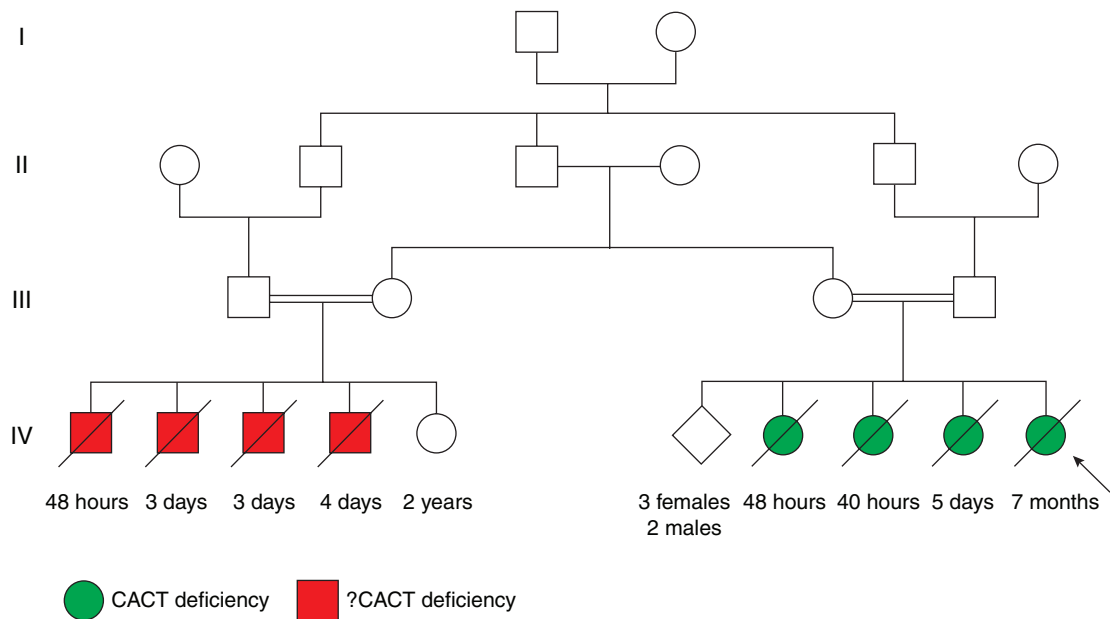


Figure 34.5 Family pedigree of the above patient with carnitine-acylcarnitine translocase deficiency is seen in two branches of the family; all previously affected neonates have died with the disease.

and levels remained over 500 between episodes. Acute elevations of uric acid have not been reported, but our experience with other disorders of fatty acid oxidation leads to a prediction that if measured they would be high [15]. Transaminase activities in the blood, both alanine and aspartate amino transferases, have been consistently elevated. Plasma concentrations of free carnitine are low, and the esterified carnitine of blood and urine elevated. In the plasma, the long chain acyl carnitine fraction was elevated. Urinary organic acid analysis may be unremarkable [1, 3], or there may be mild dicarboxylic aciduria (C6, C8, C10, C12, and unsaturated C10 and C12) [4, 14]. At times of acute episodes, 3-hydroxydicarboxylic aciduria may also be seen [16]. In response to continued fasting, failure of ketogenesis was observed, along with a relative paucity of dicarboxylic acids [1].

The advent of acylcarnitine profiles by tandem mass spectrometry has made the diagnosis of this disease considerably easier and more reliable (Table 33.1). The pattern is dominated by the elevation of long chain acylcarnitines, especially C16 and C18:1 and the deficiency of C0, free carnitine (Figure 34.6) [4]. CPT II deficiency

has an identical pattern, so enzyme assay is required for definitive diagnosis. Retrospective diagnosis of translocase deficiency has been made by the acylcarnitine profile of a neonatal blood spot (Figure 34.6) [11].

A mild phenotype has been reported [16] in the seventh born of first cousin Pakistani parents. The potential lethality of even this variant is indicated by the fact that the fifth child of this union died at three months, and the sixth had seizures, respiratory distress, and an undetectable glucose at 48 hours, and he died of ventricular tachycardia later that day. Autopsy showed severe steatosis of the myocardium, as well as of liver and kidneys. The patient reported was diagnosed by tandem mass spectrometry of a neonatal blood spot in which palmitoylcarnitine was 8.85 $\mu\text{mol/L}$ (normal, 4.82) and C2 and C0 were low. A controlled fast at four months revealed elevated free fatty acids without increase in 3-hydroxybutyrate and increased dicarboxylic and 3-hydroxydicarboxylic acids in the urine. Despite frequent feeding and attempted avoidance of fasting, he had a hypoglycemic seizure at 12 months. Cornstarch was added to the night-time regimen at two years, and he was developing normally at three years of age.

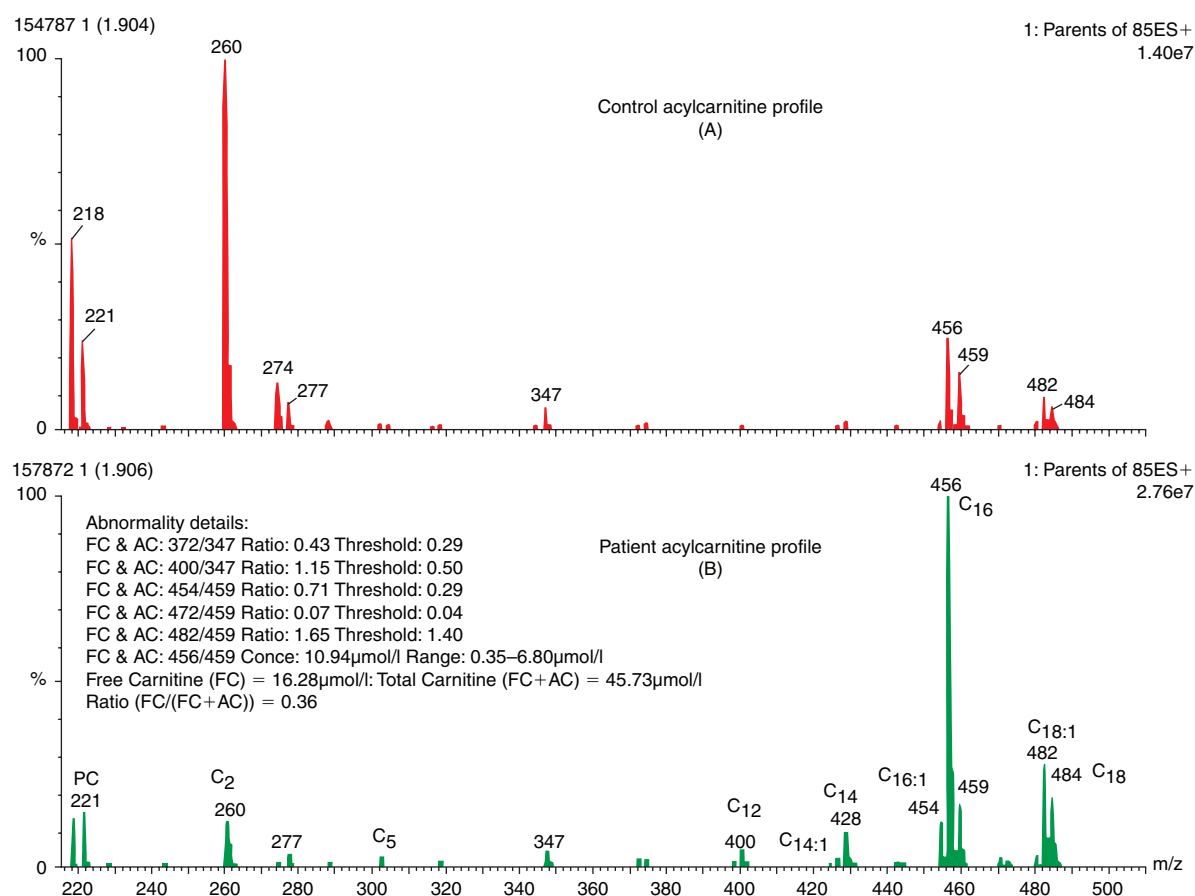


Figure 34.6 Acylcarnitine profile of blood spot of a patient with carnitine-acylcarnitine translocase deficiency and a control where carnitine was very low and long chain acylcarnitines high (peaks, 456–484). The peaks in the profile are the molecular ions (M^+) of the free carnitine peak 218, and acylcarnitine butyl esters (peaks, 260–484).

Another patient with a mild phenotype was reported at five months of age [17].

GENETICS AND PATHOGENESIS

The fundamental defect in carnitine-acylcarnitine translocase may be demonstrated in cultured fibroblasts [1]. In the first patient, activity was barely detectable at 0.8 percent of the normal mean. Prior incubation with digitonin, to increase permeability, indicated the fibroblast assay to be linear with time and protein and that with this assay the activity in the patient studied was zero [3]. In 12 patients, the activity was less than 1 percent of control in all but one [4]. In that patient, the one with the mild phenotype [16], activity ranged from 3 to 6.8 percent of normal. The enzyme can be assayed in fresh lymphocytes and in amniocytes [3, 4].

Intermediate levels of activity were found in fibroblasts of the mother and father of the first patient approximating 50 percent of control, consistent with an autosomal recessive mode of genetic transmission [1], as was consanguinity in other kindreds [3, 16]. However, overlap between control and parent levels has been observed [4]. Better discrimination has been obtained when the results were expressed as the ratio of values of pyruvate conversion to acetylcarnitine or citric acid cycle intermediates [11].

In vitro, the oxidation of fatty acids by fibroblasts or lymphocytes reveals that oxidation of long chain fatty acids, such as oleate [1] or palmitate [3] were very low, while that of octanoate was normal. Oxidation of palmitate and myristate in the patient with the milder phenotype, while abnormal, was somewhat better [16].

The differentiation of CACT from CPT II deficiency and long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency from mitochondrial trifunctional protein (MTP) deficiency continues to be ambiguous using current acylcarnitine profiling techniques either from plasma or blood spots, or in the intact cell system (fibroblasts/amniocytes). Currently, enzyme assays are required to differentiate CACT from CPT II, and LCHAD from MTP. Diagnostic elevation of unlabeled butyrylcarnitine may be detected in CACT-deficient cell lines incubated with a shorter chain fatty acid, [7-2H³] heptanoate plus L-carnitine, rather than routinely used long-chain fatty acid, [16-2H³] palmitate [18].

The pathogenesis of hypoketotic hypoglycemia and cardiomyopathy in patients with fatty acid oxidation (FAO) disorders is still poorly understood. *In vitro* studies are hampered by the lack of natural mutants to assess the effect of FAO inhibition. In addition, only a few inhibitors of FAO are known. Furthermore, most inhibitors of FAO are activating ligands of peroxisome proliferator-activated receptors (PPARs). Aminocarnitine, a carnitine analog, inhibits FAO efficiently, but does not activate PPAR, and it inhibits CPT with different sensitivities towards CPT I and CPT II, as well as CACT [19].

NADPH-cytochrome P450 reductase (CPR) is an essential component for the function of many enzymes, including microsomal cytochrome P450 (P450) monooxygenases and heme oxygenases. In mouse models, a reduced serum cholesterol level and an induction of hepatic P450s were observed, whereas hepatomegaly and fatty liver were only observed in the null model. In addition, induction of a fatty-acid translocase and suppression of carnitine-palmitoyltransferase appeared responsible for severe hepatic steatosis [20].

Prenatal diagnosis has been reported in six fetuses at risk [4]. Methodology included oxidation of fatty acids and enzyme assay in cultured amniocytes (n = 4) and chorionic villus material (n = 3). One fetus was affected. Results were confirmed in all six. Two other prenatal diagnoses of translocase deficiency have been reported [13].

The translocase enzyme, isolated from rat liver mitochondria, is 32.5 kDa [21]. Its affinity is greatest for C12–6 acylcarnitines [22]. The human translocase cDNA has been cloned [23]. It is 1.2 kb in length and codes for a 301 amino acid (32.9 kDa) protein. The gene is on chromosome 3p21.31 [24]. Mutations in the cDNA sequence have been defined in a few patients. A homozygous cytosine insertion causing a frameshift and elongation of the protein by 21 amino acids was found in a patient with a mild phenotype [23]. In a patient with severe disease, there was compound heterozygosity for two extensive deletions [25]. In a patient with severe disease from a consanguineous kindred in which seven previous siblings had had neonatal deaths, a 558 C to T transition in the cDNA led to a premature stop at amino acid 166 [4]. The transition was confirmed directly in genomic DNA of the patient and her parents [26]. In other severely affected patients, missense mutations have been reported, including G81R and R133W [27, 28]. A novel c.609-3c>g (IVS6-3c>g) mutation on the paternal allele was found in compound with a previously described c.326delG mutation on the maternal allele. Most SLC25A20 mutations have been found in a single family [29].

Deficiency of carnitine acyl translocase leads to the accumulation of the free fatty acids outside the mitochondrial matrix; long chain acylcarnitines and short chains are also found, consistent with the fact that purified translocase catalyzes the transport of short, as well as long chain acylcarnitines [21]. The long chain acylcarnitines predominate during illness following fasting-induced lipolysis. Medium and short chain esters might reflect the acyl CoA products of peroxisomal oxidation that would require transfer into the mitochondria via the translocase for final oxidation. Secondary deficiency of free carnitine would be expected to result from the excretion over time of large amounts of esterified carnitine.

The hyperammonemia was associated with normal amounts of orotic acid in the urine. This would suggest an inhibition by accumulated compounds of carbamylphosphate synthetase or acetylglutamate synthetase, as has been shown for propionic acidemia and other organic

acidemias [30]. This differs from medium chain acyl CoA dehydrogenase deficiency in which we have reported hyperammonemia and orotic aciduria [31], suggesting inhibition of the ornithine transcarbamylase step of the urea cycle.

The oxidation of long chain fatty acids is the chief source of energy during fasting for long periods and for skeletal and cardiac muscle during exercise [32]. The hepatic oxidation of long chain fats leads to ketone body production, gluconeogenesis, and maintenance of blood levels of glucose during fasting [33]. The clinical manifestations of translocase deficiency are similar to those of the infantile form of CPT II deficiency [34] in which a similar acylcarnitine profile is observed.

Dynamic acetylation and deacetylation of nuclear histones is essential for regulating the access of chromosomal DNA to transcriptional machinery. The source of acetyl-CoA for histone acetylation in mammalian cell nuclei is not clear. Acylcarnitine formed in mitochondria is transported into cytosol by carnitine/acylcarnitine translocase, and then enters nucleus, where it is converted to acetylCoA by a nuclear carnitine acetyltransferase and becomes a source of acetyl groups for histone acetylation. Genetic deficiency of the translocase markedly reduced the mitochondrial acylcarnitine-dependent nuclear histone acetylation, indicating the significance of the carnitine-dependent mitochondrial acetyl group contribution to histone acetylation [35].

TREATMENT

The ultimate courses in many of the patients described have been relentless despite treatment. However, patients with less complete defects have had milder phenotypes. Assessing the outcome of fatty acid oxidation disorders is difficult, as most are rare. For diagnosis by newborn screening, the situation is compounded: far more patients are uncovered by screening than by clinical presentation, representing a somewhat different cohort. Treatment should emphasize the avoidance of fasting and the use of intravenous glucose to prevent it. Supplemental carnitine and restriction of the intake of long chain fats are prudent. Early medical intervention in the form of intravenous 25 percent dextrose and carnitine supplementation followed by a gradual introduction of a high carbohydrate low fat diet has resulted in good clinical and biochemical response in some patients [8, 9]. The acute hyperammonemia would be expected to respond to sodium benzoate, phenylacetate, or phenylbutyrate. A trial of arginine might be effective. Cornstarch regimens appear to be useful in preventing hypoglycemia.

REFERENCES

1. Stanley CA, Hale DE, Berry GT *et al*. Brief report. A deficiency of carnitine-acylcarnitine translocase in the inner mitochondrial membrane. *N Engl J Med* 1992; **327**: 19.
2. Saudubray JM, Martin D, De Lonlay P *et al*. Recognition and management of fatty acid oxidation defects: a series of 107 patients. *J Inherit Metab Dis* 1999; **22**: 488.
3. Pande SV, Brivet M, Slama A *et al*. Carnitine-acylcarnitine translocase deficiency with severe hypoglycemia and auriculoventricular block: translocase assay in permeabilized fibroblasts. *J Clin Invest* 1993; **91**: 1247.
4. Brivet M, Boutron A, Slama A *et al*. Defects in activation and transport of fatty acids. *J Inherit Metab Dis* 1999; **22**: 428.
5. Stanley CA. Carnitine deficiency disorders in children. *Ann NY Acad Sci* 2004; **1033**: 41.
6. Wilcken B. Fatty acid oxidation disorders: outcome and long-term prognosis. *J Inherit Metab Dis* 2010; **33**: 501.
7. Al Aqeel AI, Rashid MS, Ruiters JP *et al*. A novel molecular defect of the carnitine acylcarnitine translocase gene in a Saudi patient. *Clin Genet* 2003; **64**: 163.
8. Al Aqeel AI, Rashed MS, Wanders RJ. Carnitine-acylcarnitine translocase deficiency is a treatable disease. *J Inherit Metab Dis* 1999; **22**: 271.
9. Al-Sannaa NA, Cheriyan GM. Carnitine-acylcarnitine translocase deficiency. Clinical course of three Saudi children with a severe phenotype. *Saudi Med J* 2010; **30**: 931.
10. Niezen-Koning KE, van Spronsen FJ, Ijlst L *et al*. A patient with lethal cardiomyopathy and a carnitine-acylcarnitine translocase deficiency. *J Inherit Metab Dis* 1995; **18**: 230.
11. Brivet M, Slama A, Millington DS *et al*. Retrospective diagnosis of carnitine/acylcarnitine translocase deficiency by acylcarnitine analysis in the proband. Guthrie card and enzymatic studies in the parents. *J Inherit Metab Dis* 1996; **19**: 181.
12. Brivet M, Slama A, Ogier H *et al*. Diagnosis of carnitine acylcarnitine translocase deficiency by complementation analysis. *J Inherit Metab Dis* 1994; **17**: 271.
13. Bonnet D, Martin D, De Lonlay P, Villain E. Arrhythmias and conduction defects as presenting symptoms of fatty acid oxidation disorders in children. *Circulation* 1999; **100**: 2248.
14. Ogier de Baulny H, Slama A, Touati G *et al*. Neonatal hyperammonemia caused by a defect of carnitine-acylcarnitine translocase. *J Pediatr* 1995; **127**: 723.
15. Marsden D, Nyhan WL, Barshop BA. Creatine kinase and uric acid: early warning for metabolic imbalance resulting from disorders of fatty acid oxidation. *Eur J Pediatr* 2001; **160**: 599.
16. Morris AAM, Olpin SE, Brivet M *et al*. A patient with carnitine-acylcarnitine translocase deficiency with a mild phenotype. *J Pediatr* 1998; **132**: 514.
17. Dionisi-Vici S, Garavaglia B, Bartoli A *et al*. Carnitine acylcarnitine translocase deficiency: benign course without cardiac involvement. *Pediatr Res* 1995; **37**: 147A (Abstr.).
18. Roe DS, Yang BZ, Vianey-Saban C. Differentiation of long-chain fatty acid oxidation disorders using alternative precursors and acylcarnitine profiling in fibroblasts. *Mol Genet Metab* 2006; **87**: 40.

19. Chegary M, Te Brinke H, Doolaard M *et al.* Characterization of L-aminocarnitine, an inhibitor of fatty acid oxidation. *Mol Genet Metab* 2008; **93**: 403.
20. Weng Y, DiRusso CC, Reilly AA *et al.* Hepatic gene expression changes in mouse models with liver-specific deletion or global suppression of the NADPH-cytochrome P450 reductase gene. Mechanistic implications for the regulation of microsomal cytochrome P450 and the fatty liver phenotype. *J Biol Chem* 2005; **280**: 31686.
21. Indiveri C, Tonazzi A, Palmieri F. Identification and purification of the carnitine carrier from rat liver mitochondria. *Biochem Biophys Acta* 1990; **1020**: 81.
22. Indiveri C, Tonazzi A, Prezioso G, Palmieri F. Kinetic characterization of the reconstituted carnitine carrier from rat liver mitochondria. *Biochem Biophys Acta* 1991; **1065**: 231.
23. Huizing M, Iacobazzi V, Ijlst L *et al.* Cloning of the human carnitine-acylcarnitine carrier cDNA and identification of the molecular defect in a patient. *Am J Hum Genet* 1997; **61**: 1239.
24. Viggiano L, Iacobazzi V, Marzella R *et al.* Assignment of the carnitine-acylcarnitine translocase gene (CACT) to human chromosome band 3p2131 by *in situ* hybridization. *Cytogenet Cell Genet* 1997; **79**: 62.
25. Huizing M, Wendel U, Ruitenbeek W *et al.* Carnitine-acylcarnitine carrier deficiency: identification of the molecular defect in a patient. *J Inherit Metab Dis* 1998; **21**: 262.
26. Costa C, Costa JM, Nuoffer JM *et al.* Identification of the molecular defect in a severe case of carnitine acylcarnitine carrier deficiency. *J Inherit Metab Dis* 1999; **22**: 267.
27. Invernizzi E, Garavaglia B, Parini R *et al.* Identification of the molecular defect in patients with carnitine-acylcarnitine carrier deficiency. *J Inherit Metab Dis* 1998; **21**(Suppl. 2): 56.
28. Indiveri C, Tonazzi A, Palmieri F. Identification of the carnitine carrier from rat liver mitochondria. *Biochim Biophys Acta* 1990; **1020**: 81.
29. Korman SH, Pitt JJ, Boneh A *et al.* A novel SLC25A20 splicing mutation in patients of different ethnic origin with neonatally lethal carnitine-acylcarnitine translocase (CACT) deficiency. *Mol Genet Metab* 2006; **89**: 332.
30. Coude FX, Sweetman L, Nyhan WL. Inhibition by propionyl coenzyme A of N-acetylglutamate synthetase in rat liver mitochondria. A possible explanation for hyperammonemia in propionic and methylmalonic academia. *J Clin Invest* 1979; **64**: 1544.
31. Marsden D, Sege-Petersen K, Nyhan WL *et al.* An unusual presentation of medium-chain acyl coenzyme A dehydrogenase deficiency. *Am J Dis Child* 1992; **146**: 1459.
32. Felig P, Wahren J. Fuel homeostasis in exercise. *N Engl J Med* 1975; **293**: 1078.
33. McGarry JD, Foster DW. Regulation of hepatic fatty acid oxidation and ketone body production. *Annu Rev Biochem* 1980; **49**: 395.
34. Demaugre F, Bonnefont JP, Colonna M *et al.* Infantile form of carnitine palmitoyltransferase II deficiency with hepatomuscular symptoms and sudden death: physiopathological approach to carnitine palmitoyltransferase II deficiencies. *J Clin Invest* 1991; **87**: 859.
35. Madiraju P, Pande SV, Prentki M, Madiraju SR. Mitochondrial acetylcarnitine provides acetyl groups for nuclear histone acetylation. *Epigenetics* 2009; **4**: 399.

Carnitine palmitoyl transferase I deficiency

Introduction	267	Treatment	271
Clinical abnormalities	268	References	271
Genetics and pathogenesis	269		

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia; acute episodes leading to convulsions and coma; hepatomegaly; elevated creatine phosphokinase; and deficiency of carnitine palmitoyl transferase I.

INTRODUCTION

Deficiency of carnitine palmitoyl transferase (CPT) I was first described in 1980 by Bougneres *et al.* [1, 2], in a patient who developed hypoketotic hypoglycemia and morning seizures at eight months of age. They referred to the disorder as deficiency of hepatic carnitine acyl transferase, or palmitoyl transferase, to distinguish it from the deficiency of muscular CPT, in which there is a very different phenotype of muscle pain and rhabdomyolysis, usually observed in adults after exercise [3]. They documented deficient carnitine acyl transferase activity in biopsied liver. Bonnefont *et al.* [4] clearly distinguished

CPT I and CPT II, and demonstrated that CPT I activity was deficient in fibroblasts of the original patient and two others [4–6]. CPT I (Figure 35.1) plays an integral part in the transfer of long chain fatty acids into the mitochondria, where all the enzymes of β -oxidation are located. The enzyme is situated in the outer membrane of the mitochondrion. In the reaction catalyzed, fatty acyl CoA esters are converted to carnitine esters. Medium chain and short chain fatty acids, in contrast, pass directly into mitochondria and thus do not require esterification with carnitine [7]. CPT II is situated on the inner mitochondrial membrane, catalyzes the regeneration of carnitine and the long chain fatty acyl CoAs, which then undergo β -oxidation.

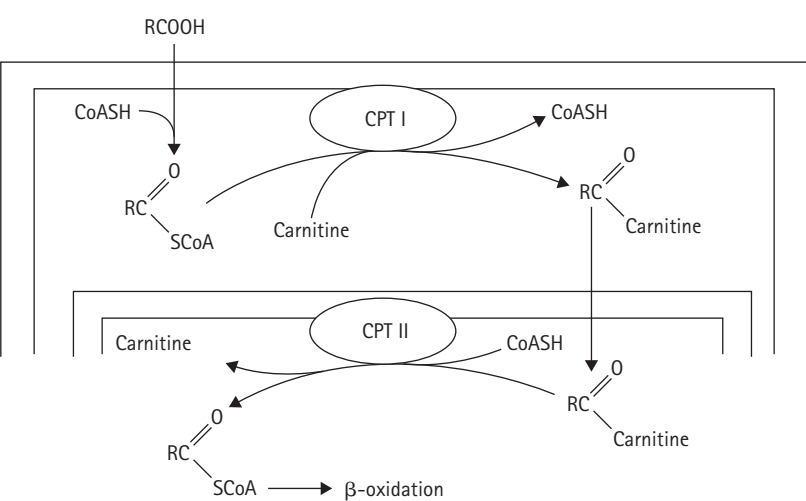


Figure 35.1 The transport of long-chain fatty acids into the mitochondrial sites of β -oxidation involves first the formation of acylcarnitine esters, catalyzed by carnitine palmitoyl transferase (CPT) I; once inside the membranes the liberation of fatty acyl CoA is catalyzed by CPT II. CPT I and II, carnitine palmitoyl transferase I and II; CoASH, coenzyme A; R, fatty acyl side chain.

Carnitine-mediated transport of fatty acids is thought to be rate-limiting in the oxidation of fats. A defect anywhere in the pathway would be expected to lead to inadequate formation of ketone bodies in response to fasting along with inadequate gluconeogenesis and hypoglycemia.

Three isoforms of CPT I have been identified [8]. Type IA or H-I, the hepatic isoform, the key regulator of fatty acid metabolism, is defective in CPT I deficiency. It transports long-chain fatty acyl-CoAs across the outer mitochondrial membrane. Affected infants have hypoketotic hypoglycemia, but often remain otherwise well. Newborn screening tests reveal elevated free carnitine (elevated C0/C16 + C18). Confirmation of the leukocyte diagnosis is accomplished by assay of the enzyme in fibroblasts. The disorder is detected by newborn screening, with variable sensitivity [9]. Type IB (M-I) is expressed in skeletal muscle. Fatty acid synthesis in the central nervous system is implicated in the control of food intake and energy expenditure. Malonyl CoA is an intermediate in this pathway. Malonyl CoA is an inhibitor of CPT I. CPT Ic knock out (KO) mice have lower body weight and food intake, and this is consistent with the function of malonyl CoA as an energy sensor. Paradoxically, CPT Ic KO mice fed a high-fat diet become obese and have decreased rates of fatty acid oxidation [10]. CPT Ic is found in the brain. CPT Ic KO mice develop obesity. Following a high fat intake, they developed severe insulin resistance, which was considered a result of increased hepatic gluconeogenesis and decreased uptake of glucose by skeletal muscle. Elevated concentrations in nonesterified fatty acids in plasma are thought to be important mediators [11]. Overexpression of CPT Ic in hypothalamus after injection of a CPT Ic adenoviral vector protects mice from obesity [12], confirming a role for CPT Ic in energy homeostasis.

Carnitine also functions as an acyl group acceptor. This has implications for therapy of disorders of fatty acid oxidation as it promotes export of acylcarnitines from the mitochondria and ultimately urinary excretion. Carnitine requirements increase under metabolic stress. Rodents fed a high fat diet accumulate acylcarnitine esters and have decreased expression of carnitine biosynthetic genes [13]. This compromises fatty acid β -oxidation. Supplementation with carnitine reversed these abnormalities and improved glucose tolerance.

Human CPT IA cDNA has been cloned [14]. It codes for 773 amino acids in a mass of 88.1 kDa. Mutations have been identified [15, 16].

CLINICAL ABNORMALITIES

This disorder presents usually in infancy, often in the second six months, with acute hypoketotic hypoglycemia during an episode of fasting brought in by an intercurrent, usually viral illness, or gastroenteritis [2, 5, 6, 17, 18]. Onset of symptoms may be neonatal or as late as 18 months. In the first family [2], a previous sister had had

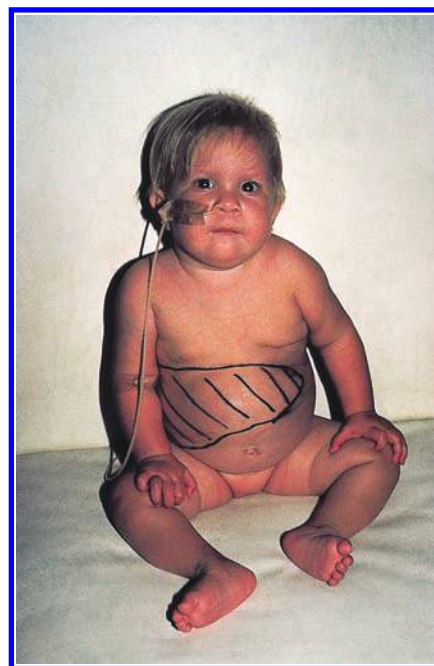


Figure 35.2 SVE: An infant with carnitine palmitoyl transferase (CPT) I deficiency [6]. She had hepatomegaly and hypoketotic hypoglycemia.



Figure 35.3 A two-year-old Saudi female with carnitine palmitoyl transferase (CPT) I deficiency. She presented with typical Reye syndrome with hepatomegaly, hypoglycemia, and hyperammonemia and very high transaminases, as well as liver failure. She was one of three siblings with the disease. A unique mutation 1950G→A resulted in a glycine 650 aspartic acid change (G650D) in the protein [26]. The family underwent preimplantation genetic diagnosis, and they have had normal children.

three hypoglycemic episodes and had died at 15 months after a 16-hour fast. The hypoglycemic episode may lead to convulsions and coma. Episodes tend to be recurrent until diagnosis and the institution of avoidance of fasting. The disease is potentially lethal. A lethal neonatal presentation has been reported [19].

The liver is usually enlarged, but soft [2]. The acute episode has often been described as Reye-like. Two patients (Figures 35.2 and 35.3) [6] developed predominantly hepatic illness, one (Fig 35.2) at ten months without documented hypoglycemia, in which hepatosplenomegaly and petechiae were associated with abnormal serum hepatocellular enzymes, prothrombin time, and partial thromboplastin time. She was thought to have disseminated intravascular coagulation and sepsis, consistent with an earlier *Klebsiella* sepsis at 2 days of age. At 14 months, she developed seizures and was found to have hypoglycemia with no ketonuria.

An interesting hepatic effect of the disease was the occurrence of acute fatty liver of pregnancy (AFLP) in a woman pregnant with each of two siblings found to have CPT I deficiency [20]. This disease then must be added to long chain hydroxyacyl CoA dehydrogenase (LCHAD) deficiency (Chapter 40) as causative of AFLP or the hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome. Hyperlipidemia has been reported during acute illness in patients with CPT I deficiency. It may serve as an alerting signal for the diagnosis [21].

Muscle biopsy may reveal lipid storage and vacuoles in electron microscopy [6], but there is no evidence of myopathy and no cardiomyopathy. This disease has been notable for its absence of cardiac symptomatology, including arrhythmias [17]. The neonatal death [19] was attributed to cardiac disease, but the only manifestations of bradycardia and arrest could reflect major systemic illness, and there were no abnormalities in cardiac or skeletal muscle at autopsy.

Renal tubular acidosis has been observed in three patients [6, 18, 22]. It may be transient. A patient displayed distal renal tubular acidosis in which there was failure to acidify the urine during spontaneous acidosis [23]. Most patients have survived and there is a tendency to decreased frequency and severity of attacks with time and with learning to avoid fasting. In general, fasting over 15 hours is required to exhaust glycogen stores and call on fatty acid oxidation.

Cognitive deficit, or its absence, depends generally on the severity of the initial hypoglycemic episode, but many patients described have been neurologically impaired [18]. In patients with residual neurologic deficit, the electroencephalograph (EEG) may show focal slowing or spike discharges, and neuroimaging may show cerebral atrophy [6]. There may be a continuing seizure disorder [22]. Linear growth and anthropometric development tend to be normal.

The creatine phosphokinase (CK) in the blood may be elevated during acute episodes [24], and this has been attributed to the MM isozyme, but the alanine transaminase (ALT) and aspartate transaminase (AST) were even more elevated; the levels of these hepatic enzymes were also elevated in other patients during acute hypoglycemic episodes [6, 23]. Blood sugar levels during the acute attack have been reported at 0.3, 0.9, 1.2,

1.3, and 1.6 mmol/L [2, 5, 6, 22, 23]. In one patient, there were recurrent Reye-like episodes without hypoglycemia, although there was improvement in lethargy on the administration of glucose [22].

Organic acid analysis of the urine is notable for the absence of dicarboxylic aciduria and hydroxydicarboxylic aciduria [2, 6, 18], as well as the absence of elevation of 3-hydroxybutyric and acetoacetic acids in the urine at times of fasting and hypoglycemia. Plasma levels of carnitine may be normal or elevated [24, 25]. Levels of both free and total carnitine may be elevated. Fractionation of the esterified carnitine of the urine reveals only acetylcarnitine. Tandem mass spectrometry of the blood reveals an absence of long chain acyl carnitines (C16, C18, C18:1) [18, 26]. The ratio of C0 to C16 + 118 is useful in diagnosis [27]. In three patients the ratio ranged from 175 to 2000, a range of 2–32 was observed in control infants. Higher values were found in older infants, but there was no overlap of patients and controls.

The histologic examination of the liver has revealed microvesicular and macrovesicular steatosis [6, 24]. Muscle biopsy may show sarcolemmal and interfibrillar accumulation of glycogen, as well as the presence of lipid [6]. A hematophagocytic syndrome has been observed in a patient with otherwise clinically typical CPT I deficiency, who developed a brain abscess with candida [28]. This syndrome has also been observed in propionic acidemia and lysinuric protein intolerance.

GENETICS AND PATHOGENESIS

The disorder is transmitted in an autosomal recessive fashion. Affected children of both sexes have been observed with normal parents. Consanguinity has been documented [2, 24]. In one extended inbred Hutterite kindred, a brother and sister and their second cousin were affected [23]. The disorder is rare [18], but there were nine patients in the Paris experience of Saudubray *et al.* [17] of 107 patients with abnormalities of fatty acid oxidation.

The enzymatic defect in CPT I is most carefully documented by measuring the production of labeled palmitoyl-carnitine from methyl-labeled carnitine and palmitoyl CoA in fibroblast homogenates, in which the integrity of mitochondrial membranes is preserved [5, 24]. Testing with and without malonyl CoA distinguishes CPT I, which is inhibited by malonyl CoA, from CPT II, which is not. Reported activity has ranged from 9 to 23 percent of control. CPT I appears to determine the overall rate of oxidation of fatty acids in the liver. Some patients have been documented to have deficiency of CPT I in liver, while activity in muscle was normal [29]. Enzyme assay for confirmation of the diagnosis of CPT I deficiency has been facilitated by the addition of cyanide to the reaction mixture to inhibit the activity of enzymes downstream of CPT I which otherwise degrade the palmitoylcarnitine formed in the assay [30]. Quantification is by tandem mass spectrometry.

In earlier assays, fibroblasts from patients with CPT I deficiency incubated with labeled palmitate accumulate labeled palmitoyl CoA, but not palmitoyl carnitine [31]. The fibroblasts of CPT I-deficient patients display defective overall oxidation of long chain fatty acids, such as palmitate, whereas oxidation of octanoate and succinate is normal [5, 32, 33]. Similarly, the conversion of ^3H -palmitate to $^3\text{H}_2\text{O}$ in fibroblast monolayers was markedly deficient [33]. These observations are consistent with the failure of CPT I-deficient cells to transport long chain fatty acids into mitochondria, while medium chain compounds are transported normally.

Immunochemical studies have been carried out with antibodies against CPT I and CPT II [34, 35]. CPT I has been demonstrated immunochemically in rat liver and kidney. Immunochemically, CPT I is absent in muscle and heart, which provided early evidence for the presence of the muscle-specific isoform. The hepatic isoform approximates 88 kDa in size, while that of muscle is approximately 82 kDa; both variants are found in heart.

Parents of affected siblings have been found to be intermediate in levels of CPT I activity in fibroblasts, consistent with heterozygosity. Prenatal diagnosis has not been reported, but preimplantation genetic diagnosis has yielded normal offspring (Figure 35.3).

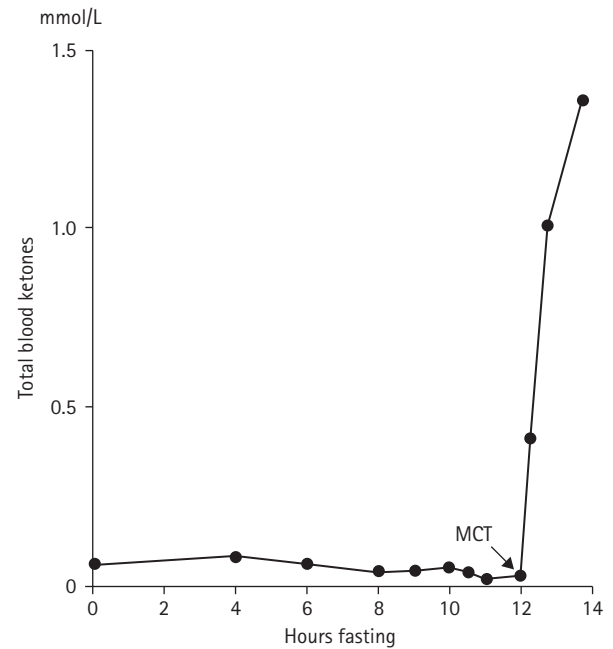


Figure 35.4 Absence of ketogenesis with fasting to hypoglycemia in carnitine palmitoyl transferase (CPT) I deficiency and the brisk ketogenic response to medium-chain triglyceride (MCT). The blood sugar also rose after MCT.

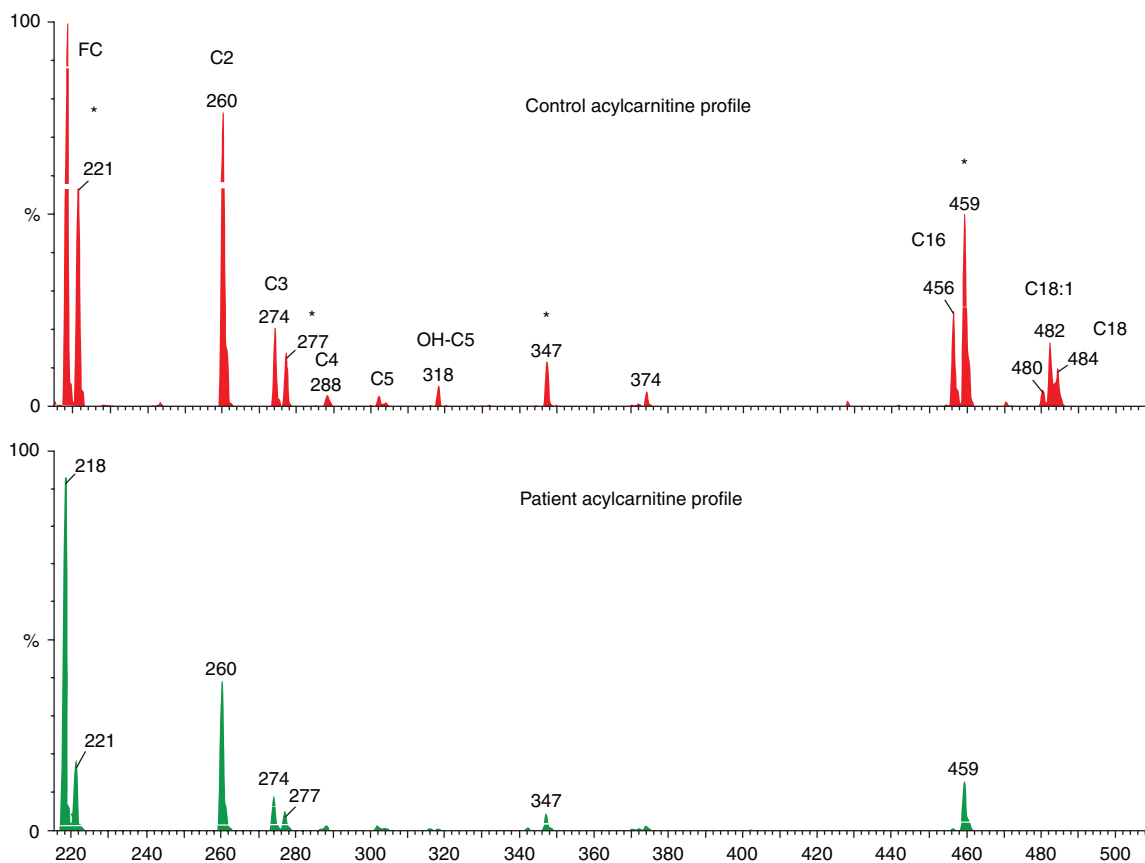


Figure 35.5 Acylcarnitine profiles assayed in blood spots of patients with carnitine palmitoyl transferase (CPT) I deficiency. The very high free carnitine and almost absent long chain acylcarnitines (peaks 456–484) are illustrated. The peaks in the profile are the molecular ions (M^+) of the free carnitine peak 218 and acylcarnitine butyl esters (peaks 260–484).

The fatty acid transport protein FAT/CD36 found in the plasma membrane may also contribute to the regulation of fatty acid oxidation. FAT/CD36 KO mice are unaffected by sulfo-N-succinimidyleste which inhibits palmitate transport across the plasma membrane [36]. Regulation of mitochondrial fatty acid oxidation is particularly relevant during the metabolic demands of muscle contraction. The CPT 1A gene on chromosome 11q13.1-13.5 [37] is expressed in liver, kidney, pancreas, ovary, leukocytes, and fibroblasts [16]. The gene spans 60 kb and contains 20 exons. The first mutation described [15] was a missense (D454G) change, which when expressed, had 2 percent of wild-type activity. Other mutations identified [16] include Q100X, which would predict an early truncation of the protein, H414V, and Y498C, which affect highly conserved sequences in the catalytic core of the enzyme. An 8-kb deletion encompassing intron 14 to exon 17 led to loss of the mRNA [16]. The rarity of the disease and the general severity of phenotype have made genotype-phenotype correlations difficult, but the mutation leading to P479L resulted in a late onset disease in which there was proximal myopathy. Homozygosity for the 1436 (C>T) mutation was identified [38] in patients with deficient CPT 1A enzyme.

In a Japanese newborn, two novel mutations, p.R446X and p.G719D were found [39].

Greenberg and colleagues [40] have addressed the high frequency of the P479L variant in Canada among the Inuit and First Nation families [41]. In the Greenland Inuit, the frequency of this allele was 0.73 in contrast to its complete absence in the nonaboriginal population. Clinical manifestations have tended to be minor or absent. Studies of fatty acid oxidation in fibroblasts revealed residual activity of the enzyme which should be sufficient to permit flux through the mitochondrial oxidation system.

The p.P479L was associated with elevated high density lipoprotein (HDL) and apoA-1 levels in plasma [41]. It has been suggested that the polymorphism might protect against atherosclerosis.

TREATMENT

The major element in management is the studied avoidance of fasting. In the presence of intercurrent infection or other cause of vomiting or anorexia in which the oral route is excluded, the provision of intravenous glucose is essential. Reduction of the intake of long chain fats appears prudent. Medium chain triglycerides may be substituted (Figure 35.4). The ideal concentration of glucose is at least 10 percent, and 25 percent has been recommended [42], along with intravenous carnitine. With recovery from the acute episode, a high carbohydrate low-fat diet supplemented with medium chain triglycerides is introduced [28]. Acute hyperammonemia may be managed by infusion of L-arginine. Cornstarch regimens are useful in preventing hypoglycemia.

REFERENCES

1. Bougnères PF, Saudubray JM, Marsac C *et al*. Decreased ketogenesis due to deficiency of hepatic carnitine acyl transferase. *N Engl J Med* 1980; **302**: 123.
2. Bougnères PF, Saudubray JM, Marsac C *et al*. Fasting hypoglycemia resulting from hepatic carnitine palmitoyl transferase deficiency. *J Pediatr* 1981; **98**: 742.
3. Bank WJ, DiMauro S, Bonilla E *et al*. A disorder of muscle lipid metabolism and myoglobinuria: absence of carnitine palmitoyl transferase. *N Engl J Med* 1975; **292**: 443.
4. Bonnefont JP, Ogier H, Mitchell G *et al*. Heterogeneity des déficits en palmitoyl carnitine transferase. *Arch Fr Pediatr* 1985; **42**: 613.
5. Demaugre F, Bonnefont JP, Mitchell G *et al*. Hepatic and muscular presentations of carnitine palmitoyl transferase deficiency: two distinct entities. *Pediatr Res* 1988; **24**: 308.
6. Bonnefont JP, Haas R, Wolff J *et al*. Deficiency of carnitine palmitoyltransferase I. *J Child Neurol* 1989; **4**: 198.
7. McGarry JD, Wright PH, Foster DW. Hormonal control of ketogenesis. *J Clin Invest* 1975; **55**: 1202.
8. Weis BC, Esser V, Foster DW *et al*. Rat heart expresses two forms of mitochondrial carnitine palmitoyltransferase I. *J Biol Chem* 1994; **269**: 18712.
9. Wilcken B. Fatty acid oxidation disorders: outcome and long-term prognosis. *J Inher Metab Dis* 2010; **33**: 501.
10. Wolfgang MJ, Kurama T, Dai Y *et al*. The brain-specific carnitine palmitoyltransferase-1c regulates energy homeostasis. *Proc Natl Acad Sci USA* 2006; **103**: 7282.
11. Gao XF, Chen W, Kong XP *et al*. Enhanced susceptibility of Cpt1c knockout mice to glucose intolerance induced by a high-fat diet involves elevated hepatic gluconeogenesis and decreased skeletal muscle glucose uptake. *Diabetologia* 2009; **52**: 912.
12. Dai Y, Wolfgang MJ, Cha SH *et al*. Localization and effect of ectopic expression of CPT1c in CNS feeding centers. *Biochem Biophys Res Commun* 2007; **359**: 469.
13. Noland RC, Koves TR, Seiler SE *et al*. Carnitine insufficiency caused by aging and overnutrition compromises mitochondrial performance and metabolic control. *J Biol Chem* 2009; **284**: 22840.
14. Britton CH, Schultz RA, Zhang B *et al*. Human liver mitochondrial carnitine palmitoyltransferase I: characterization of its cDNA and chromosomal localization and partial analysis of the gene. *Proc Natl Acad Sci USA* 1995; **92**: 1984.
15. Ijlst L, Mandel H, Oostheim W *et al*. Molecular basis of hepatic carnitine palmitoyltransferase I deficiency. *J Clin Invest* 1998; **102**: 527.
16. Gobin S, Bonnefont JP, Prip-Buus C *et al*. Organization of the human liver carnitine palmitoyltransferase 1 gene (CPT1A) and identification of novel mutations in hypoketotic hypoglycaemia. *Hum Genet* 2002; **111**: 179.
17. Saudubray JM, Martin D, De Lonlay P *et al*. Recognition and management of fatty acid oxidation defects: a series of 107 patients. *J Inher Metab Dis* 1999; **22**: 488.

18. Brivet M, Boutron A, Slama A *et al.* Defects in activation and transport of fatty acids. *J Inherit Metab Dis* 1999; **22**: 428.
19. Invernizzi F, Burlina AB, Donadio A *et al.* Case report. Lethal neonatal presentation of carnitine palmitoyltransferase I deficiency. *J Inherit Metab Dis* 2001; **24**: 601.
20. Innes AM, Seargeant LE, Balachandra K *et al.* Hepatic carnitine palmitoyltransferase I deficiency presenting as maternal illness in pregnancy. *Pediatr Res* 2000; **47**: 43.
21. Worthington H, Olpin SE, Blumenthal I *et al.* Hyperlipidaemia due to carnitine palmitoyltransferase I deficiency. *J Inherit Metab Dis* 2007; **30**: 104.
22. Falik-Borenstein ZC, Jordan SC, Saudubray J-M *et al.* Renal tubular acidosis in carnitine palmitoyltransferase type 1 deficiency. *N Engl J Med* 1992; **327**: 24.
23. Bergman A, Donckerwolcke R, Duran M *et al.* Rate-dependent distal renal tubular acidosis and carnitine palmitoyltransferase I deficiency. *Pediatr Res* 1994; **36**: 582.
24. Haworth JC, Demaugre F, Booth FA *et al.* Atypical features of the hepatic form of carnitine palmitoyltransferase deficiency in a Hutterite family. *J Pediatr* 1992; **121**: 553.
25. Stanley CA, Sunaryo F, Hale DE *et al.* Elevated plasma carnitine in the hepatic form of carnitine-palmitoyltransferase-1 deficiency. *J Inherit Metab Dis* 1992; **15**: 785.
26. Al Aqeel A, Rashid MS, Wanders RJ. Carnitine palmitoyltransferase deficiency. Three affected siblings in a Saudi family. *Saudi Med J* 2001; **22**: 1025.
27. Fingerhut R, Roschinger W, Muntau A *et al.* Hepatic carnitine palmitoyltransferase I deficiency: acylcarnitine profiles in blood spots are highly specific. *Clin Chem* 2001; **47**: 1763.
28. Al-Aqeel AI, Rashed MS, Ijst L *et al.* Phenotypic variability of carnitine palmitoyl transferase I deficiency (CPT I) with novel molecular defect in Saudi Arabia. *Am J Hum Genet* 2002; **71**: 412.
29. Tein I, Demaugre F, Bonnefont J-P, Saudubray J-M. Normal muscle CPT₁ and CPT₂ activities in hepatic presentation patients with CPT1 deficiency in fibroblasts: tissue-specific isoforms of CPT₁. *J Neurol Sci* 1989; **92**: 229.
30. van Vlies N, Ruiter JP, Doolaard M *et al.* An improved enzyme assay for carnitine palmitoyl transferase I in fibroblasts using tandem mass spectrometry. *Mol Genet Metab* 2007; **90**: 24.
31. Schaefer J, Jackson S, Taroni F *et al.* Characterization of carnitine palmitoyltransferases in patients with a carnitine palmitoyltransferase deficiency: implications for diagnosis and therapy. *J Neurol Neurosurg Psychiatry* 1997; **62**: 169.
32. Saudubray JM, Coude FX, Demaugre F *et al.* Oxidation of fatty acids in cultured fibroblasts: a model system for the detection and study of defects in oxidation. *Pediatr Res* 1982; **16**: 877.
33. Mitchell G, Saudubray JM, Pelet A *et al.* The effect of D-carnitine on palmitate oxidation in cultured fibroblasts. *Clin Chim Acta* 1984; **143**: 23.
34. Kolodziej MP, Crilly PJ, Corstorphine CG, Zammit VA. Development and characterization of polyclonal antibody against rat liver mitochondrial overt carnitine palmitoyltransferase (CPT I). Distinction of CPT I from CPT II and of isoforms of CPT I in different tissues. *Biochem J* 1992; **282**: 415.
35. Demaugre F, Bonnefont J-P, Cepanec C *et al.* Immunoquantitative analysis of human carnitine palmitoyltransferase I and II defects. *Pediatr Res* 1990; **27**: 497.
36. Holloway GP, Jain SS, Bezaire V *et al.* FAT/CD36-null mice reveal that mitochondrial FAT/CD36 is required to upregulate mitochondrial fatty acid oxidation in contracting muscle. *Am J Physiol Regul Integr Comp Physiol* 2009; **297**: R960.
37. Gellera C, Verderio E, Floridia G *et al.* Assignment of the human carnitine palmitoyltransferase II gene to chromosome 1a32. *Genomics* 1994; **24**: 195.
38. Park JY, Narayan SB, Bennett MJ. Molecular assay for detection of the common carnitine palmitoyltransferase 1A1436(C>T) mutation. *Clin Chem Lab Med* 2006; **44**: 1090.
39. Tsuburaya R, Sakamoto O, Arai N *et al.* Molecular analysis of a presymptomatic case of carnitine palmitoyl transferase I (CPT I) deficiency detected by tandem mass spectrometry newborn screening in Japan. *Brain Dev* 2010; **32**: 409.
40. Greenberg CR, Dilling LA, Thompson GR *et al.* The paradox of the carnitine palmitoyl transferase type Ia P479L variant in Canadian Aboriginal populations. *Mol Genet Metab* 2009; **96**: 201.
41. Rajakumar C, Ban MR, Cao H *et al.* Carnitine palmitoyltransferase IA polymorphism P479L is common in Greenland Inuit and is associated with elevated plasma apolipoprotein A-I. *J Lipid Res* 2009; **50**: 1223.
42. Longo N, Amat di San Filippo C, Pasquali M. Disorders of carnitine transport and the carnitine cycle. *Am J Med Genet C Semin Med Genet* 2006; **142C**: 77.

Carnitine palmitoyl transferase II deficiency, lethal neonatal

Introduction	273	Treatment	276
Clinical picture	273	References	276
Genetics and pathogenesis	275		

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia, enlarged polycystic kidneys, hepatomegaly, microcephaly, prominent forehead, over folded helices, cardiomegaly, cardiac arrhythmias, elevated long chain acylcarnitine esters, and profoundly decreased activity of carnitine palmitoyl transferase II (CPT II).

INTRODUCTION

This distinct phenotype of CPT II deficiency, first described by Hug and colleagues in 1989 [1, 2] provides another example of effects of an inherited disorder of metabolism on early intrauterine organogenesis. In addition to hypoketotic hypoglycemia and hepatomegaly, affected infants have cardiomegaly and cardiac arrhythmias. They also have dysmorphic features and massively enlarged cystic kidneys [1–3]. The brain is also dysplastic and cystic [4–7]. Death in infancy has been uniform.

The activity of CPT II (see Figure 35.1 in Chapter 35) is markedly reduced in many tissues, including cultured fibroblasts [4–7]. This leads to a characteristic profile of acylcarnitines in which there is elevation of all long chain species, particularly C16, C18:2, C18:1, and C18 (Figure 36.1). This feature permits the detection of the disease by expanded programs of newborn screening by tandem mass spectrometry (MS/MS) [8].

The gene is located on chromosome 1p32. A small number of mutations has been detected, including an 11-bp duplication [9], a 2-bp deletion [6], and compound heterozygosity for two truncating mutations [10].

CLINICAL PICTURE

The disease typically presents in the first days of life with lethargy, hypotonia, or seizures, and the infant is found to

have hypoglycemia, hyperammonia, or both [1–4]. The concentration of ammonia may exceed 1000 μmol/L [4]. Hypoglycemia is classically hypoketotic, and the urine test for ketones is usually negative [4]. All but one of reported neonatal onset patients have died by one month of age (Figures 36.2, 36.3, 36.4, 36.5, 36.6, and 36.7) [11]. A few patients have had a more classic disorder of fatty acid oxidation phenotype with onset of hypoketotic hypoglycemia at five to ten months following an intercurrent illness that led to prolonged fasting [10–12]. Nevertheless, most of these patients or their siblings have died in infancy. There have been two notable exceptions [10, 12].

The massively enlarged polycystic kidneys are a major manifestation of neonatal onset patients (Figure 36.2) [3, 4]. Pregnancy may be complicated by oligohydramnios [4]. The kidneys may be visible, readily palpable, and shown to be polycystic by ultrasound [4]. Prenatal diagnosis has been accomplished by fetal ultrasonography [13–15]. Hyperkalemia may signal rapidly progressive renal failure.

Hepatomegaly is also characteristic [1, 2, 4] and liver size may increase progressively. Aminotransferase levels may be elevated. Hepatic calcifications have been seen on ultrasound [10]. Histologic examination has revealed lipid vacuoles in hepatocytes [4, 6].

Cardiomegaly may be associated with arrhythmias [1–3, 6] and cardiac failure. Lipid accumulation has been documented in cardiac myocytes [4, 6]. Left ventricular

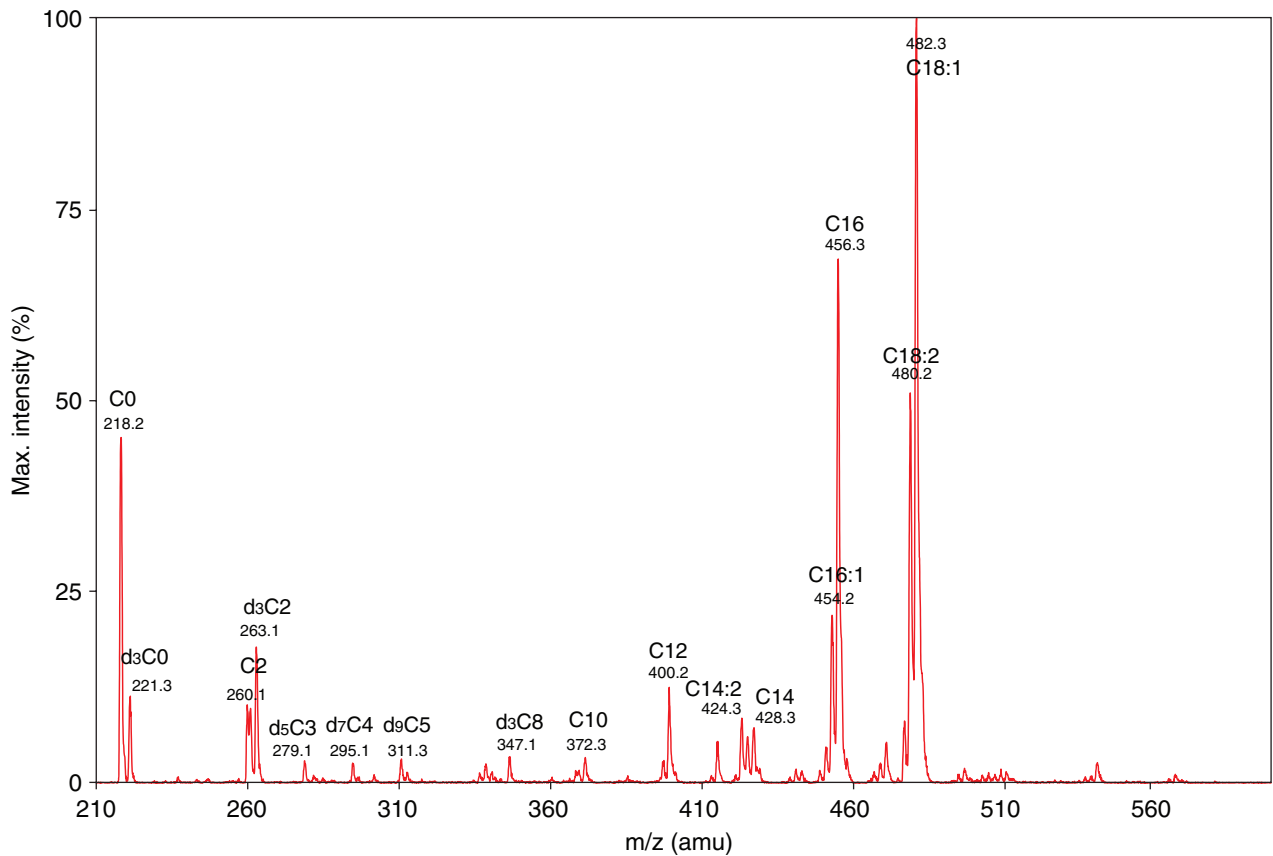


Figure 36.1 Acylcarnitine profile of a neonate with lethal carnitine palmitoyl transferase (CPT) II deficiency.



Figure 36.2 Baby T: A neonate with carnitine palmitoyl transferase (CPT) II deficiency who died a few days later at 12 days of age. The enormous kidneys visible in the abdomen were recognized as polycystic on prenatal ultrasound. The liver was also palpable 2 cm below the costal margin. She had hypotonia and microcephaly. She also had cardiomyopathy and pronounced cerebral ventriculomegaly.



Figure 36.3 Baby T: The ears were low set and rotated posteriorly. The forehead appeared long and sloped backwards.

wall and septum were hypertrophied. Skeletal muscle also showed depositions of lipid [4, 6]. A patient with a myopathic presentation who died at 34 days had deficiency of CPT in muscle [16]. Creatine kinase activity may be increased [15].

Dysmorphic features have included microcephaly, a high sloping forehead, flat occiput, over folded helices, long tapering fingers and toes, extra digital creases on fingers 2 to 4 bilaterally, widely spaced nipples, hypoplastic toenails,



Figure 36.4 Baby T: The helix came to a point just under the nasal catheter.



Figure 36.5 Baby T: The nose appeared bulbous.



Figure 36.6 BR: A neonate with carnitine palmitoyl transferase (CPT) II deficiency who died at 14 days of age. She had a high backward sloping forehead and a bulbous nose. Renal failure is evident in facial edema. Nipples were widely spaced. Polycystic kidneys were evident on ultrasonography: but this was subtle and the kidneys were not palpable, even during hyperammonemic coma.



Figure 36.7 The ear was not low, but it was posteriorly rotated. The patient presented on the first day of life with hypoglycemia and hyperammonemia, which responded promptly to the infusion of arginine.

and contractures of the knees, elbows, and small joints of the hands [4]. Another infant [15] had a high arched narrow palate.

Cystic dysplasia of the brain has also been reported [4, 7], as has polymicrogyria and intracerebral hemorrhage [6]. Cysts may be visible on cranial ultrasonography [4, 7]. On electron microscopy, paraventricular cysts were lined with dense gliosis. There were glial heterotopias at the base of the brain [4].

GENETICS AND PATHOGENESIS

CPT II activity has been documented to be decreased in cultured fibroblasts and tissue homogenates. Enzyme activity is generally measured in the forward and reverse directions [17–19], and CPT I activity has been demonstrated by its malonyl CoA sensitivity in the forward reaction. Profound deficiency of CPT II activity found in this phenotype [12, 20] contrasts with the partial deficiency that characterizes the CPT II deficiency that presents with intermittent rhabdomyolysis, in adolescence or adulthood (see below) [21].

Long chain fatty acids require a carnitine transport system in order to gain entrance to the mitochondrial matrix where β -oxidation takes place (Chapter 32). CPT II is located on the inner side of the inner mitochondrial membrane. It catalyzes the conversion of long chain acylcarnitine esters, like palmitoylcarnitine, to free carnitine and the corresponding CoA ester, such as palmitoyl CoA.

Concentrations of acylcarnitines are elevated in blood and tissues [4, 7, 10]. In addition to the elevations of especially C16 to C18 (Figure 36.1), the ratios of C16/C8:1 and C18:1/C8:1 are enormously elevated. This pattern may also be seen in carnitine-acylcarnitine transferase (CACT) deficiency.

Urinary organic acids are usually normal. A few patients have had medium-chain dicarboxylic aciduria, but without hexanoylglycine or suberylglycine. Plasma and urinary total carnitine may be elevated, particularly the esterified fraction. Free carnitine in blood and tissue is usually normal, or even elevated, but it may decrease rapidly [4].

Oxidation of palmitic acid and myristic acid in cultured fibroblasts is decreased [10, 12].

Information on molecular genetics is just beginning. The 11-bp duplication was found [9] in two siblings reported by Witt *et al.* [13]. The two truncating mutations were found in an Ashkenazi Jewish infant who died on the third day of life [10]. A number of other mutations found in Ashkenazi infants included the 2-bp deletion and a missense mutation in exon 4 [15]. Compound heterozygosity for these mutations has been found in Ashkenazi patients with the adult form of CPT II deficiency [22].

TREATMENT

The neonatal presentation has led uniformly to early death. A potential exception is a patient who was living at the time of report at 14 months following treatment of acute decompensation with exchange transfusion, along with a long-term high caloric diet supplemented with medium chain triglyceride [23]. This patient had a unique mutation, a 24-bp deletion leading to deletion of amino acids 179–186 and substitution of phenylalanine for leucine at 178.

REFERENCES

- Hug GS, Berry H, Bove K. Carnitine palmitoyl transferase (CPT): deficiency of CPT II but not of CPT I with reduced total and free carnitine but increased acylcarnitine. *Pediatr Res* 1989; **25**(Suppl.): 115A.
- Hug GS, Bove KE, Soukup S. Lethal neonatal multiorgan deficiency of carnitine palmitoyltransferase II. *N Engl J Med* 1991; **325**: 1862.
- Zinn ABZ, Kraus F, Strohl C *et al.* Carnitine palmitoyltransferase B (CPT B) deficiency: a heritable cause of neonatal cardiomyopathy and dysgenesis of the kidney. *Pediatr Res* 1991; **29**(Suppl.): 73A.
- North KN, Hoppel CL, De Girolami U *et al.* Lethal neonatal deficiency of carnitine palmitoyltransferase II associated with dysgenesis of the brain and kidneys. *J Pediatr* 1995; **127**: 414.
- Land JM, Mistry S, Squier M *et al.* Neonatal carnitine palmitoyltransferase-2 deficiency: a case presenting with myopathy. *Neuromuscul Disord* 1995; **5**: 129.
- Taroni FG, Cavadini P, Baratta S *et al.* Lethal carnitine palmitoyltransferase (CPT) II deficiency in newborns: a molecular-genetic study. *Am J Hum Genet* 1994; **55**(Suppl.): A245.
- Pierce MR, Pridjian G, Morrison S, Pickoff AS. Fatal carnitine palmitoyltransferase II deficiency in a newborn: new phenotypic features. *Clin Pediatr* 1999; **38**: 13.
- Albers S, Marsden D, Quackenbush E *et al.* Detection of neonatal carnitine palmitoyltransferase II deficiency by expanded newborn screening with tandem mass spectrometry. *Pediatrics* 2001; **107**: E103.
- Gellera CW, Verderio E, Cavadini P *et al.* Molecular study of lethal neonatal carnitine palmitoyltransferase II (CPT II) deficiency. *Am J Hum Genet* 1992; **51**(Suppl.): A168.
- Vladutiu GD, Quackenbush EJ, Hainline BE *et al.* Lethal neonatal and severe late infantile forms of carnitine palmitoyltransferase II deficiency associated with compound heterozygosity for different protein truncation mutations. *J Pediatr* 2002; **141**: 734.
- Elpeleg ON, Joseph A, Gutman A. Editorial correspondence. *J Pediatr* 1994; **124**: 160.
- Elpeleg ON, Joseph A, Branski D *et al.* Recurrent metabolic decompensation in profound carnitine palmitoyltransferase II deficiency. *J Pediatr* 1993; **122**: 917.
- Witt DRT, Santa-Maria M, Packman S *et al.* Carnitine palmitoyl transferase-type 2 deficiency: two new cases and successful prenatal diagnosis. *Am J Hum Genet* 1991; **49**(Suppl.): A109.
- Sharma R, Perszyk AA, Marangi D *et al.* Lethal neonatal carnitine palmitoyltransferase II deficiency: an unusual presentation of a rare disorder. *Am J Perinatol* 2003; **20**: 25.
- Elpeleg ON, Hammerman C, Saada A *et al.* Antenatal presentation of carnitine palmitoyltransferase II deficiency. *Am J Med Genet* 2001; **102**: 183.
- Land JM, Mistry S, Squier W *et al.* Neonatal carnitine palmitoyltransferase deficiency: a case with a muscular presentation. In: Coates P (ed.). *New Developments in Fatty Acid Oxidation. (Progress in Clinical Biological Research)*. New York: Wiley-Liss, 1992: **375**: 309.
- Hoppel CL, Tomec RJ. Carnitine palmitoyltransferase. Location of two enzymatic activities in rat liver mitochondria. *J Biol Chem* 1972; **247**: 832.
- Taroni F, Verderio E, Dworzak F *et al.* Identification of a common mutation in the carnitine palmitoyltransferase II gene in familial recurrent myoglobinuria patients. *Nat Genet* 1993; **4**: 314.
- McGarry JD, Woeltje KF, Kuwajima M, Foster DW. Regulation of ketogenesis and the renaissance of carnitine palmitoyltransferase. *Diabet Metab Rev* 1989; **5**: 271.
- Demaugre F, Bonnefont JP, Colonna M *et al.* Infantile form of carnitine palmitoyltransferase II deficiency with hepatomuscular symptoms and sudden death. Physiopathological approach to carnitine palmitoyltransferase II deficiencies. *J Clin Invest* 1991; **87**: 859.
- DiMauro S, DiMauro PM. Muscle carnitine palmitoyltransferase deficiency and myoglobinuria. *Science* 1973; **182**: 929.
- Taggart RT, Smail D, Apolito C, Vladutiu GD. Novel mutations associated with carnitine palmitoyltransferase II deficiency. *Hum Mutat* 1999; **13**: 210.
- Smeets RJ, Smeitink JA, Semmekrot BA *et al.* A novel splice site mutation in neonatal carnitine palmitoyl transferase II deficiency. *J Hum Genet* 2003; **48**: 8.

Carnitine palmitoyl tranferase II deficiency, late onset

Introduction	277	Treatment	279
Clinical abnormalities	277	References	279
Genetics and pathogenesis	278		

MAJOR PHENOTYPIC EXPRESSION

Muscular, pain, rhabdomyolysis, and myoglobinuria triggered by exercise, cold, or febrile illness; elevation of creatine kinase; and deficient activity of carnitine palmitoyltransferase II in muscle.

INTRODUCTION

Carnitine palmitoyl tranferase II (CPT II) deficiency was the first disorder of mitochondrial fatty acid oxidation to be reported. In 1973, DiMauro and DiMauro [1] reported an adolescent adult onset disease with predominantly myopathic symptomatology. The disease is thought to be the most common disease of fatty acid oxidation [2, 3].

CPT II deficiency has also been found in two rare infantile forms ([Chapter 36](#)): one a fatal neonatal form in which there is hypoglycemia and multiorgan involvement [4–6] and a severe form that may begin after the neonatal period and present with hypoketotic hypoglycemia, encephalopathy, hepatomegaly, or cardiomyopathy [7, 8].

The diagnosis of CPT2 deficiency has classically been made by assay of the enzyme in muscle. Currently, it may also be made by mutational analysis. The common myopathic phenotype results frequently from a C to T transition at position 439 leading to a serine 113 change to leucine (S113L) [9]. When homozygous, this mutation yields an enzyme with 15–25 percent of control activity. Genotype–phenotype correlation is evident in comparison to this picture with those of the severe neonatal forms which result in no demonstrable enzyme activity and no immunochemically detectable enzyme protein [10]. Compound heterozygosity for S113L and a more severe mutation has led to intermediate phenotypes with intermediate levels of enzyme activity [11].

The occurrence of symptomatic first-degree relatives of patients with CPT II deficiency has led to the idea that heterozygotes may be symptomatic. The gene may function

as a dominant. In a study of 34 symptomatic patients whose enzyme activity was lower than 25 percent below the normal mean, mutational analysis was consistent with the presence of a single mutation in five [12]. Enzymatic activity in these five was 29 percent of control, whereas in those in whom two mutations were found it was 11 percent of control.

CLINICAL ABNORMALITIES

The symptomatology of CPT II deficiency is predominantly that of acute muscle injury with onset usually in teenage or young adulthood [13]. Symptoms are those of pain in muscles or myoglobinuria accompanied by a pronounced rise in the level of creatine kinase (CK) in the blood. Symptoms may be induced by exercise or by cold [13, 14]. Immersion in cold water, even only of the lower legs in the ocean, is an effective trigger. Episodes are recurrent [1]. They may also be induced by prolonged fasting [15] and by infection [16, 17]. A high-fat, low-carbohydrate diet may lead to muscle aches and increased CK [13]. The disease may be a cause of unexplained CK elevation without clinical symptoms [16]. In a recent series of 28 patients, myalgia induced by exercise was the most common manifestation, occurring in 96 percent. Myoglobinuria was absent in 21 percent of patients [18]. Pain may be limited to muscles exercised. Pain in the legs is most common. Another presentation is recurrent abdominal pain. The effect of the disease on muscle may be manifest in progressive myopathic weakness, particularly in proximal muscles of the shoulder

and hip [19]. An unusual manifestation reported [16] was acute respiratory failure requiring resuscitation. Another patient had lethal cardiac arrhythmia [17].

Myoglobinuria may lead to renal failure [14, 17]. Patients with CPT II deficiency may develop malignant hyperthermia associated with surgery or anesthesia [20, 21]. An unusual presentation was recurrent pancreatitis associated with prolonged exercise and a high-fat diet [22].

Levels of CK in the blood may be very high, e.g. 31,000 [15]. Aldolase and transaminase activities may also be elevated, as may uric acid [14]. Rhabdomyolysis may also be accompanied by hyperkalemia, hyperphosphatemia, and hypocalcemia [17].

Fasting which induces muscle aches and CK elevation may be noted to be without ketosis or ketonuria [23], but these responses to fasting may be normal [15]. A high-carbohydrate diet has been observed to suppress the acute myopathic response to fasting and exercise [24]. Administration of medium chain triglycerides (MCT) leads to the normal increase in levels of acetoacetate and 3-hydroxybutyrate in the blood and ketonuria [13, 15].

Electron microscopy has revealed droplets of lipid in muscle [13]. Triglyceride concentrations in the blood may be elevated [23, 24], and so may those of free fatty acids [23]. Necrotic muscle fibers may be detected during acute attacks [14].

Stable isotope metabolism and indirect calorimetry revealed normal long-chain fatty acid oxidation at rest, but severe impairment during prolonged low intensity exercise [25] in heterozygotes, as well as compounds of two different mutations.

Insulin resistance has been documented in a woman homozygous for the S113L mutation [26].

The diagnosis of CPT II deficiency has classically been made by enzyme assay of biopsied muscle, but currently it is most commonly suggested by the acylcarnitine profile of whole blood or plasma (see Table 32.1 in Chapter 32) [27]. The characteristic pattern is that of an elevation of C16 and C18:1; acetylcarnitine (C2) is not elevated. The ratio of C16 and C18:1/C2 in serum detected all of known patients with CPT II deficiency studied. The ratio in a patient was $0.24 \pm 0.014 \mu\text{mol/L}$ (range, 0.08–0.56); in controls, the ratio was 0.032 ± 0.011 [27]. This permits the detection of this disease by newborn screening, although the elevations are less pronounced in dried blood spots than in plasma [27]. This pattern will not distinguish CPT II deficiency from that of carnitine/acylcarnitine translocase (CACT) deficiency (Chapter 33), but that distinction becomes a problem only with newborn screening and patients with the infantile CPT II disease (Chapter 36).

GENETICS AND PATHOGENESIS

The nature of the molecular defect was initially established by assay of the activity of the enzyme in biopsied muscle [1,

14]. In patients with this late onset disease, there was little correlation between the level of enzyme activity and the degree of clinical severity; although patients with the lethal neonatal disease (Chapter 36) have much less enzyme activity. Activity in muscle from the late onset form ranges from 15 to 20 percent of control levels, while the neonatal forms display less than 10 percent of activity. Enzyme activity can also be documented in cultured fibroblasts and peripheral blood leukocytes [28, 29].

The gene was mapped by human–hamster somatic cell hybridization technique to chromosome 1 [30]. Fluorescent *in situ* hybridization localized the site to 1p32 [30]. The DNA sequence codes for a protein of 658 amino acids and contains an N-terminal leader peptide [31]. The gene consists of five exons and 20 kb of DNA [9].

At least 25 disease-producing missense mutations have been reported [32, 33]. The most common C to T transition at nucleotide 439 causes the S133L change in more than 50 percent of mutant alleles [9, 10]. All other mutations are rare [10, 34]. Genotype–phenotype correlation has been elusive, but patients with the neonatal disease have been located in exons 4 and 5 [33]. These patients display inefficient oxidation of palmitate, less than 10 percent of control, whereas cells with adult type mutations range from 45 to 70 percent. Heterozygosity for a cysteine for arginine (R503C) change in a highly conserved region of exon 4 was found in a typically severely myopathic affected patient [21] in whom CPT II activity was 42 percent of normal in cultured lymphoblasts and 13 percent of control in muscle. Complete sequence analysis of the gene uncovered no other mutations.

Expression of the disease as an autosomal dominant appears to be favored by the tetramer structure of the enzyme in which there may be dominant negative interaction of normal and abnormal subunits in the formation or function of the tetramer. It is also possible that other genetic factors influence expression. There are polymorphisms in the gene, and a patient had a haplotype with a polymorphism in exon 5, whereas her asymptomatic father who also had the R503C mutation did not have the haplotype. The same mutation was found in a patient with malignant hyperthermia and no myopathy [20]. Patients with single dominant mutations E454X and D213G had symptoms of cramping or rhabdomyolysis only after exercise, as might be expected for a dominant negative effect [25].

A 26-bp deletion (1237AG) was found in compound with S113L in four Ashkenazi Jewish patients with late onset disease [35].

Screening for mutations in CPT II can be done on muscle, leukocytes, lymphoblasts, and fibroblasts, and it has been adapted to spotted whole blood on filter paper [36]. Restriction fragment length polymorphism (RFLP) screening for S113L would miss a significant number of patients; therefore, tandem mass spectrometry (MS/MS) quantification of the acylcarnitine profile is currently the preferred method.

TREATMENT

Patients with CPT II deficiency tend to avoid symptomatology by altering their lifestyles. Avoidance of immersion in cold water and avoidance of strenuous exercise appear prudent. A high-carbohydrate, low-fat diet and the avoidance of fasting are recommended. Supplemental carnitine does not appear to help.

Consideration has been given to pharmacologic approaches to the promotion of fatty acid oxidation [37]. Fibrates employed as hypolipidemic agents in the treatment of hypertriglyceridemia are activators of the nuclear receptor, PPAR α , which transactivates genes of lipid and lipoprotein metabolism [38, 39] by upregulating expression of genes encoding mitochondrial enzymes. Bezafibrate, one of these drugs employed as a clinical hypolipidemic, was studied in an attempt to correct fatty acid oxidation in cultured fibroblasts derived from patients with CPT II deficiency [38]. It was found that bezafibrate led to a time- and dose-dependent increase in CPT II mRNA from 47 to 66 percent and of enzyme activity from 54 to 135 percent of control, as well as a return to normal of rates of oxidation at ^3H -myristate.

REFERENCES

1. Dimauro S, Dimauro PM. Muscle carnitine palmitoyltransferase deficiency and myoglobinuria. *Science* 1973; **182**: 929.
2. Bennett MJ, Rinaldo P, Strauss AW. Inborn errors of mitochondrial fatty acid oxidation. *Crit Rev Clin Lab Sci* 2000; **37**: 1.
3. McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur J Biochem* 1997; **244**: 1.
4. Hug G, Bove KE, Soukup S. Lethal neonatal multiorgan deficiency of carnitine palmitoyltransferase II. *N Engl J Med* 1991; **325**: 1862.
5. North KN, Hoppel CL, De Girolami U *et al*. Lethal neonatal deficiency of carnitine palmitoyltransferase II associated with dysgenesis of the brain and kidneys. *J Pediatr* 1995; **127**: 414.
6. Zinn AB, Zurcher VL, Kraus F *et al*. Carnitine palmitoyltransferase B (CPT B) deficiency: a heritable cause of neonatal cardiomyopathy and dysgenesis of the kidney. *Pediatr Res* 1991; **29**: 73A.
7. Demaugre F, Bonnefont JP, Colonna M *et al*. Infantile form of carnitine palmitoyltransferase II deficiency with hepatomuscular symptoms and sudden death. Physiopathological approach to carnitine palmitoyltransferase II deficiencies. *J Clin Invest* 1991; **87**: 859.
8. Elpeleg ON, Joseph A, Branski D *et al*. Recurrent metabolic decompensation in profound carnitine palmitoyltransferase II deficiency. *J Pediatr* 1993; **122**: 917.
9. Taroni F, Verderio E, Dworzak F *et al*. Identification of a common mutation in the carnitine palmitoyltransferase II gene in familial recurrent myoglobinuria patients. *Nat Genet* 1993; **4**: 314.
10. Bonnefont JP, Taroni F, Cavadini P *et al*. Molecular analysis of carnitine palmitoyltransferase II deficiency with hepatocardiomyocardial expression. *Am J Hum Genet* 1996; **58**: 971.
11. Vladutiu GD, Quackenbush EJ, Hainline BE *et al*. Lethal neonatal and severe late infantile forms of carnitine palmitoyltransferase II deficiency associated with compound heterozygosity for different protein truncation mutations. *J Pediatr* 2002; **141**: 734.
12. Taggart C, Apolito D, Small GD *et al*. Mutation analysis of carnitine palmitoyl transferase II deficiency. *Hum Mutat* 1999; **13**: 210.
13. Engel WK, Vick NA, Glueck CJ *et al*. A skeletal-muscle disorder associated with intermittent symptoms and a possible defect of lipid metabolism. *N Engl J Med* 1970; **282**: 697.
14. Brownell AK, Severson DL, Thompson CD *et al*. Cold induced rhabdomyolysis in carnitine palmitoyl transferase deficiency. *Can J Neurol Sci* 1979; **6**: 367.
15. Hostetler KY, Hoppel CL, Romine JS *et al*. Partial deficiency of muscle carnitine palmitoyltransferase with normal ketone production. *N Engl J Med* 1978; **298**: 553.
16. Bertorini T, Yeh YY, Trevisan C *et al*. Carnitine palmitoyl transferase deficiency: myoglobinuria and respiratory failure. *Neurology* 1980; **30**: 263.
17. Kelly KJ, Garland JS, Tang TT *et al*. Fatal rhabdomyolysis following influenza infection in a girl with familial carnitine palmitoyl transferase deficiency. *Pediatrics* 1989; **84**: 312.
18. Deschauer M, Wieser T, Zierz S. Muscle carnitine palmitoyltransferase II deficiency: clinical and molecular genetic features and diagnostic aspects. *Arch Neurol* 2005; **62**: 37.
19. Meola G, Bresolin N, Rimoldi M *et al*. Recessive carnitine palmitoyl transferase deficiency: biochemical studies in tissue cultures and platelets. *J Neurol* 1987; **235**: 74.
20. Vladutiu G, Taggart RT, Smail D. A carnitine palmitoyl transferase II (CPT2) arg503cys mutation confers malignant hyperthermia and variable myopathy. *Am J Hum Genet* 1998; **63**(Suppl.): 20.
21. Vladutiu GD, Bennett MJ, Smail D. A variable myopathy associated with heterozygosity for the R503C mutation in the carnitine palmitoyltransferase II gene. *Mol Genet Metab* 2000; **70**: 134.
22. Tein I, Christodoulou J, Donner E, McInnes R. Carnitine palmitoyltransferase II deficiency: a new cause of recurrent pancreatitis. *J Pediatr* 1994; **124**: 938.
23. Bank WJ, Dimauro S, Bonilla E *et al*. A disorder of muscle lipid metabolism and myoglobinuria. Absence of carnitine palmitoyl transferase. *N Engl J Med* 1975; **292**: 443.
24. Cumming WJ, Hardy M, Hudgson P *et al*. Carnitine-palmitoyl-transferase deficiency. *J Neurol Sci* 1976; **30**: 247.
25. Orngreen MC, Duno M, Ejstrup R *et al*. Fuel utilization in subjects with carnitine palmitoyltransferase 2 gene mutations. *Ann Neurol* 2005; **57**: 60.
26. Haap M, Thamer C, Machann J *et al*. Metabolic characterization of a woman homozygous for the Ser113Leu missense mutation in carnitine palmitoyl transferase II. *J Clin Endocrinol Metab* 2002; **87**: 2139.

27. Gempel K, Kiechl S, Hofmann S *et al.* Screening for carnitine palmitoyltransferase II deficiency by tandem mass spectrometry. *J Inherit Metab Dis* 2002; **25**: 17.
28. Angelini C, Fredo L, Battistella P *et al.* Carnitine palmitoyl transferase deficiency: clinical variability, carrier detection, and autosomal-recessive inheritance. *Neurology* 1981; **31**: 883.
29. Schaefer J, Jackson S, Taroni F *et al.* Characterisation of carnitine palmitoyltransferases in patients with a carnitine palmitoyltransferase deficiency: implications for diagnosis and therapy. *J Neurol Neurosurg Psychiatry* 1997; **62**: 169.
30. Gellera C, Verderio E, Floridia G *et al.* Assignment of the human carnitine palmitoyltransferase II gene (CPT1) to chromosome 1p32. *Genomics* 1994; **24**: 195.
31. Finocchiaro G, Taroni F, Rocchi M *et al.* cDNA cloning, sequence analysis, and chromosomal localization of the gene for human carnitine palmitoyltransferase. *Proc Natl Acad Sci USA* 1991; **88**: 661.
32. Verderio E, Cavadini P, Montermini L *et al.* Carnitine palmitoyltransferase II deficiency: structure of the gene and characterization of two novel disease-causing mutations. *Hum Mol Genet* 1995; **4**: 19.
33. Thuillier L, Rostane H, Droin V *et al.* Correlation between genotype, metabolic data, and clinical presentation in carnitine palmitoyltransferase 2 (CPT2) deficiency. *Hum Mutat* 2003; **21**: 493.
34. Martin MA, Rubio JC, De Bustos F *et al.* Molecular analysis in Spanish patients with muscle carnitine palmitoyltransferase deficiency. *Muscle Nerve* 1999; **22**: 941.
35. Taggart RT, Smail D, Apolito C *et al.* Novel mutations associated with carnitine palmitoyltransferase II deficiency. *Hum Mutat* 1999; **13**: 210.
36. Smail D, Gambino L, Boles C *et al.* Rapid, cost-effective gene mutation screening for carnitine palmitoyltransferase II deficiency using whole blood on filter paper. *Clin Chem* 1999; **45**: 2035.
37. Djouadi F, Bonnefont JP, Thuillier L *et al.* Correction of fatty acid oxidation in carnitine palmitoyl transferase 2-deficient cultured skin fibroblasts by bezafibrate. *Pediatr Res* 2003; **54**: 446.
38. Berger J, Moller DE. The mechanisms of action of PPARs. *Annu Rev Med* 2002; **53**: 409.
39. Vamecq J, Latruffe N. Medical significance of peroxisome proliferator-activated receptors. *Lancet* 1999; **354**: 141.

Medium chain acyl CoA dehydrogenase deficiency

Introduction	281	Treatment	286
Clinical abnormalities	282	References	287
Genetics and pathogenesis	285		

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia, cardiomyopathy cardiac arrhythmia, sudden infant death syndrome, myopathy, hyperammonemia, hyperuricemia, elevated creatine kinase, dicarboxylic aciduria, elevated C6 and C8 acylcarnitine, deficient activity of medium chain acyl CoA dehydrogenase, and mutation in the ACADM gene, especially A985G.

INTRODUCTION

Medium chain acyl CoA dehydrogenase (MCAD) (Figure 38.1 and Table 38.1) deficiency is the classic disorder of fatty acid oxidation, and it is the most common. Occurring in an estimated 1 in 6000–10,000 Caucasian births, the disease was nevertheless first described in 1983 [1, 2] an index of the difficulties, even today, in detecting disorders of fatty acid oxidation [3].

Disorders of fatty oxidation display two general types of presentation. The first, hypoketotic hypoglycemia, is the clinical picture of Reye syndrome. In fact, it is now clear that most patients who appear to have Reye syndrome have an inborn error of metabolism, the most common being MCAD deficiency and ornithine transcarbamylase deficiency (Chapter 24) [4, 5]. The other presentation reflects the chronic disruption of muscle function with symptoms relevant to myopathy or cardiomyopathy, including weakness, hypotonia, congestive heart failure,

Table 38.1 The acyl CoA dehydrogenases and their substrate specificities

Enzyme	Substrate chain length	Deficiency disease
Short Chain (SCAD)	C4-6	Rarely symptomatic
Medium Chain (MCAD)	C6-12	Common (1: 10,000)
Very Long Chain (VLCAD)	C14-20	Uncommon

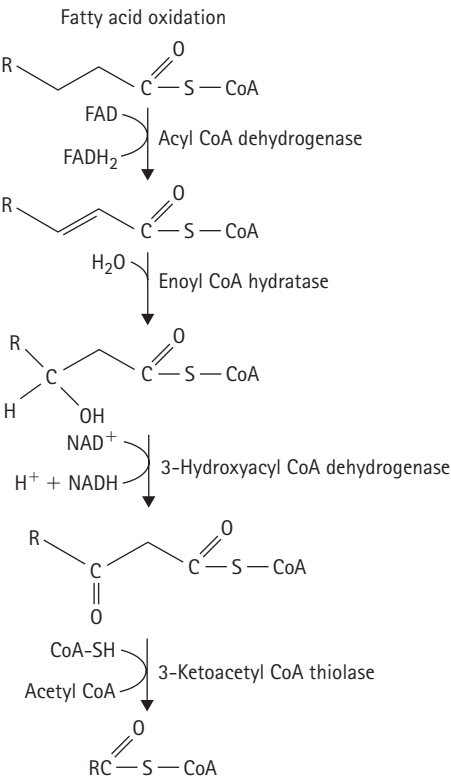


Figure 38.1 The pathway of β -oxidation of fatty acids begins with the acyl CoA dehydrogenase step.

or arrhythmia. Both types of presentation may be seen in the same family or even in the same individual. Another presentation is with the sudden infant death syndrome (SIDS) [6–9]. We and others have been able to make retrospective diagnoses of MCAD in infants who had died of SIDS by retrieval of neonatal screening blood spots after making the diagnosis of MCAD deficiency in a subsequent sibling and assay for the common mutation in the DNA or for octanoylcarnitine. The introduction of the tandem mass spectrometric analysis of acylcarnitines has greatly facilitated the diagnosis of this and other disorders of fatty acid oxidation, and its application to the screening of newborns is a major addition to preventive medicine. This could prevent many further examples of SIDS due to MCAD deficiency.

The gene was cloned in 1986 [10] and assigned to chromosome 1. A majority of Caucasian patients have the A985 gene mutation.

CLINICAL ABNORMALITIES

Episodic illness usually occurs first between six months and two years, usually following fasting for 12 hours or more as a consequence of intercurrent infectious disease (Figures 38.2, 38.3, and 38.4). The episode may be ushered in with vomiting or lethargy, or it may begin with a seizure. It is progressive rapidly to coma [11]. Patients are typically hypoglycemic. Hypoglycemia with a simultaneous negative urine test for ketones is very helpful in diagnosis.

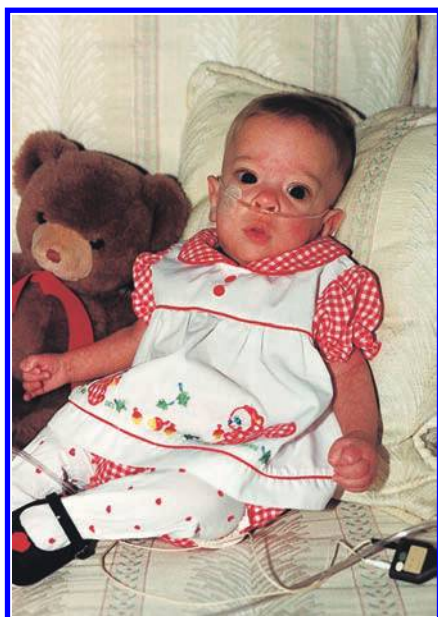


Figure 38.2 SE: An eight-month-old infant with medium chain acyl CoA dehydrogenase deficiency, 2 weeks before her first episode of hypoglycemia. She had multiple episodes of hypoglycemia over the next year.



Figure 38.3 SE: At 26 months. These episodes were behind her, but she was still receiving treatment with carnitine and cornstarch.



Figure 38.4 KB: A one-year-old patient with medium chain acyl CoA dehydrogenase (MCAD) deficiency. She presented at seven months with a life-threatening episode of illness. Her sibling died in the first days of life of sudden infant death syndrome and was documented retrospectively to have MCAD deficiency. Once diagnosed, this patient had not had another episode requiring admission to hospital. Nevertheless, the dangerous nature of this disease is signified by the fact that she died at home during sleep without evident prior illness.

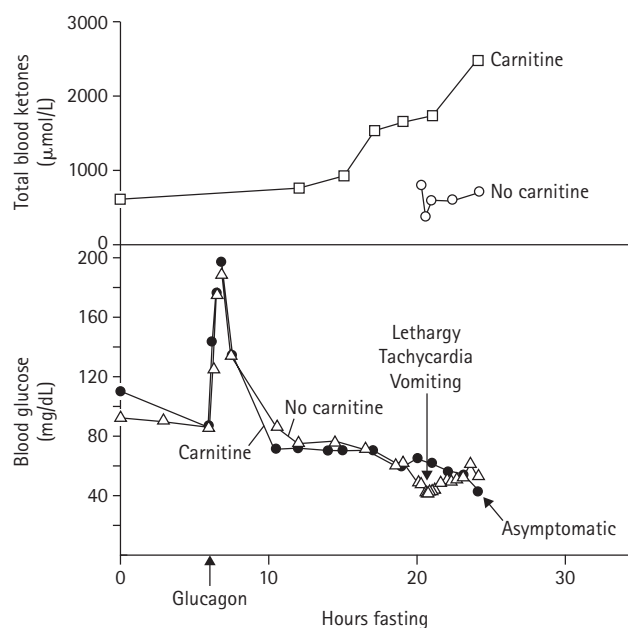


Figure 38.5 Fasting ketogenesis in a patient with medium chain acyl CoA dehydrogenase (MCAD) deficiency. A second fast was initiated after initiation of treatment with carnitine, and although she again became hypoglycemic, she had a better ketogenic response, did not have hypoglycemia until 24 hours, and did not develop clinical evidence of illness.

However, although these patients are documentably hypoketotic (Figure 38.5), the urine often contains some ketones at times of acute illness, so this can be misleading [12]. Hepatomegaly is usually present at the time of the acute illness. Liver biopsy at the time reveals abundant deposits of lipid in microvesicular pattern [13]. This and hyperammonemia have often led to a diagnosis of Reye syndrome [2, 4, 14–16]. Cerebral edema and herniation have been reported in an acute lethal episode [15]. In at least one patient [14], a documented accompanying orotic aciduria permitted fulfillment of published criteria for a diagnosis of ornithine transcarbamylase deficiency, but assay of this enzyme in biopsied liver revealed normal activity. This Reye-like presentation is often the first manifestation of disease in this disorder [17].

During the intervals between episodes of illness, patients typically appear completely well. There is no muscle weakness. However, some patients are impressively hypotonic and display a reluctance to exercise or poor muscle strength. Clinical myopathy or cardiomyopathy is unusual in this condition, particularly early [17], but these problems may develop in any patient with a disorder of fatty acid oxidation. Acute cardiac arrhythmia may be seen at the time of episodic illness.

The issue of arrhythmia in MCAD deficiency has received less than optimal attention in the literature, possibly because so many patients diagnosed early do so well. Rice and colleagues [18] reported a 3-day-old patient

with ventricular tachyarrhythmia and torsades de pointes refractory to medication that ultimately responded to extracorporeal life support and intravenous carnitine. We have seen arrhythmia visualized on monitor in the intensive care unit during an initial hypoglycemic episode. We have also wondered about all those infants with SIDS, like the ones that have been documented on retrieved blood spots once a subsequent sibling has been diagnosed. Arrhythmia appears to be a more logical cause of SIDS than hypoketotic hypoglycemia.

Iafolla and colleagues [19, 20] have assembled data on 120 patients with MCAD deficiency referred for diagnostic testing. The mean age of onset was 12 months and the range was 2 days to 6.5 years. Of 120 patients, 23 died before the diagnosis was made; 12 siblings of patients had died previously, 11 diagnosed as SIDS, and one as Reye syndrome. Emergency care or admission to hospital was required at onset in 95 percent of patients. Initial symptoms were lethargy in 84 percent, vomiting in 66 percent, encephalopathy in 49 percent, and respiratory arrest in 48 percent. Cardiac arrest was the initial presentation in 36 percent and sudden death in 18 percent. Seizures were present in 43 percent and hepatomegaly in 44 percent.

The acute severity of presentation contrasts with the fact that there were no deaths in 97 surviving patients for an average of 2.6 years following diagnosis. Most of our patients have not had a second episode requiring admission to hospital. On the other hand, in follow-up data on 73 patients older than two years of age [19], there was appreciable long-term morbidity. Twenty-nine percent were judged developmentally delayed; of these, 12 had global developmental disability. Another seven had behavioral problems. Seizure disorder was present in 14 percent and attention deficit disorder in 11 percent.

Key elements of the clinical chemistry in suggesting a disorder of fatty acid oxidation are markedly elevated levels of uric acid and creatine kinase (CK) [14, 21–23]. These values are elevated only during the acute episodes of illness when they are very high. Uric acid concentrations are often over 10 mg/dL and have been as high as 20 mg/dL. CK may be over 1000 U/L. These data, along with evidence of large amounts of urate in the urine, indicate that the mechanism is cellular breakdown. Hyperuricemia and high levels of CK have been observed in patients with rabies [24]. In patients suspected of having a disorder of fatty acid oxidation, it is important to order these two alerting tests specifically, as they are usually omitted from panels of clinical chemical tests in children's hospitals.

Carnitine deficiency is the rule in this disease and may be helpful in suggesting the diagnosis. Concentrations of free-carnitine are very low in plasma. Levels are also low in tissues, but muscle biopsy is not commonly available in this disorder. Levels of esterified carnitine are very high in the urine, and this is the mechanism of the secondary carnitine deficiency. In any condition in which the CoA esters of carboxylic acids accumulate, esterification with carnitine takes place, and carnitine esters are preferentially excreted

in the urine. This serves a detoxification function, but it also depletes supplies of carnitine, leading to a second mechanism of impaired fatty acid oxidation. Ratios of ester to free-carnitine tend to be high in blood, as well as urine, but we find this less useful than the actual levels of free-carnitine in the plasma and esterified carnitine in the urine.

Medium-chain dicarboxylic aciduria is the hallmark of the organic acid profile in the urine of the patient with this disease, and it may be diagnostic at the time of the acute episode [15]. The typical pattern is that of large amounts of the dicarboxylic acids, adipic (C6), suberic (C8), and sebacic (C10), as well as the glycine conjugates of hexanoic acid and suberic acid. The very large elevation of suberylglycine may be diagnostic [25, 26]. Dicarboxylic acids, as long as C12 (dodecanedioic) acid, may be elevated, and omega-1 oxidation yields the hydroxy acids, 5-hydroxy-hexanoic and 7-hydroxy-octanoic acids [27]. Normal infants and children excrete large amounts of dicarboxylic acids with fasting, but the attendant ketosis is mirrored in very large excretions of 3-hydroxybutyric acid and acetoacetic acid. In contrast, in patients with disorders of fatty acid oxidation, the ratio of dicarboxylic acids to the sum of these two compounds is greater than 1 [2]. Unfortunately, these diagnostic features disappear from the urine with the disappearance of the acute episode, so that by the time the patient is referred for study, the organic acid analysis of the urine is usually completely normal.

Fasting under controlled conditions will reproduce the typical pattern of dicarboxylic acids in the urine. We have developed a protocol for the systematic investigation of patients suspected of having disorders of fatty acid oxidation (Chapter 32). It is now never necessary in MCAD deficiency because of the number of simple, less invasive tests that are available. Figure 38.5 illustrates its use in a patient diagnosed prior to the development of these tests. At the time that she developed hypoglycemia after 20 hours, she excreted diagnostic quantities of suberylglycine. The flat curve for the blood levels of acetoacetic and

3-hydroxybutyric acids illustrates the impaired ketogenesis, along with the development of hypoglycemia and clinical evidence of illness.

The diagnosis of MCAD deficiency in the absence of illness or fasting has been facilitated by the development of a sensitive method of gas chromatography-mass spectrometry (GCMS) using a stable isotope dilution that permits the measurement of the glycine conjugates hexanoylglycine, suberylglycine, and phenylpropionylglycine, even in the normal individuals [26]. The amounts found in patients with MCAD deficiency during remission are often large enough to be diagnostic. Phenylpropionylglycine excretion depends on the conjugation of a product of intestinal microbial metabolism, and we have found that it is usually absent during the acute episode, when most patients are receiving antibiotic therapy. Also patients not colonized by the anaerobic clostridia that produce phenylpropionic acid will not be distinguishable in this way [28].

The modern approach to the diagnosis that may be used in remission, as well as during illness, is to examine the blood for specific carnitine esters by tandem mass spectrometry (MS/MS) [27, 29–31]. The test is usually carried out on blood spots in programs of newborn screening. For definitive diagnosis, the analysis of plasma is preferable. Octanoylcarnitine is the compound on which reliance is usually placed (Figure 38.6 and Table 32.1 in Chapter 32). Hexanoylcarnitine is also useful, and the ratio of C8 to C8:1 is often the best discriminator [29, 32, 33]. Normal newborns have levels under 0.22 $\mu\text{mol/L}$ of octanoylcarnitine (C8). The mean of 16 patients with MCAD deficiency was 8.4 (range, 3.1–28.3) $\mu\text{mol/L}$ [29] and of 35 patients 3.0 (0.4–21.8) $\mu\text{mol/L}$ [32]. A level over 0.3 has been considered a diagnostic criterion for MCAD deficiency. With time and depletion of carnitine, levels of C8 carnitine decrease in patients with MCAD deficiency. For this test to be successful it is sometimes necessary to administer some carnitine.

The pathology of MCAD deficiency is predominantly

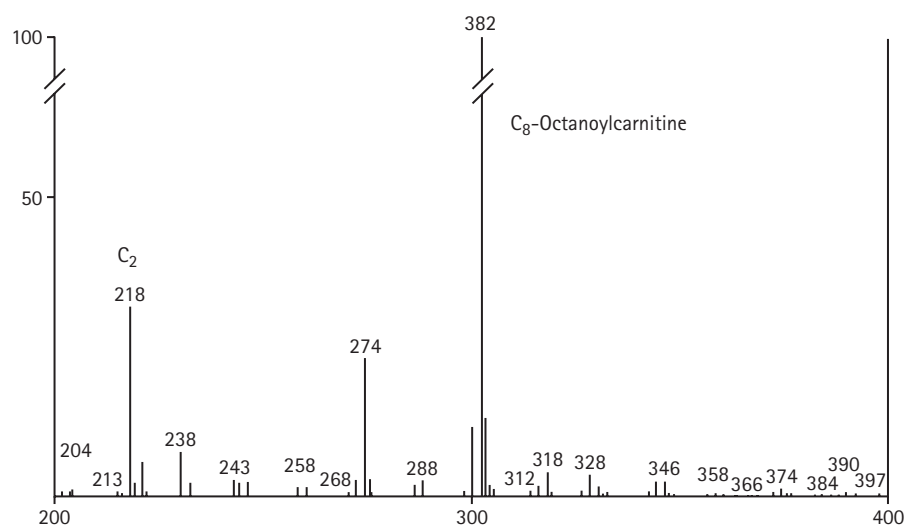


Figure 38.6 Acylcarnitine profile of the plasma of a patient with medium chain acyl CoA dehydrogenase (MCAD) deficiency. C8 is octanoylcarnitine. Illustration provided by Jon Gangoiti of the University of California, San Diego.

that of the liver [13, 17, 34] in which microvesicular and macrovesicular deposits of fat are typical. Deposition of lipids has also been observed in the kidneys and heart [19]. Cerebral edema has been described in the neuropathology of MCAD deficiency, but this has rested on very little evidence. In the paper usually quoted [2], a two-year-old patient, one of three patients reported, died and had cerebral edema and herniation on autopsy. Our teenager with MCAD deficiency [14] certainly had increased intracranial pressure, but she had hyperammonemia (235 $\mu\text{mol/L}$), a well-recognized cause of cerebral edema, and her encephalopathy resolved with the level of ammonia. Cerebral edema was also reported in two patients by Duran *et al.* [35]. In the series of 120 patients with MCAD deficiency, cerebral edema was recorded in 14 of 23 studied at autopsy [19]. Cerebral edema was also recorded by Bennett *et al.* [25] in an infant with MCAD deficiency who died suddenly in her sleep.

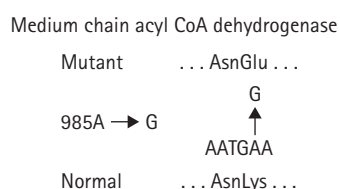


Figure 38.7 The mutation at nucleoside 985. The AT to G change specifies a change from the basic lysine to the acidic glutamic acid.

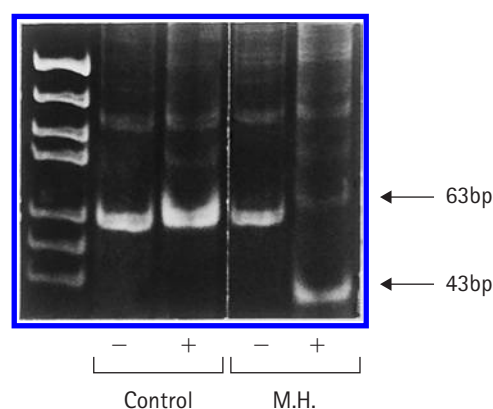


Figure 38.8 Detection of the common mutation in medium chain acyl CoA dehydrogenase (MCAD) deficiency; autoradiography of DNA fragments after electrophoresis on a 3 percent agarose gel. This preparation was made by Dr Karin Sege-Peterson with the method of Matsubara *et al.* [38], in which polymerase chain reaction (PCR) with a mismatched primer permitted a restriction site in the mutant, but not in the normal. Thus, in the mutant, a 63-bp fragment contains the site which leads to a 43-bp fragment when treated with *NcoI*. In this illustration, – and + signify without and with *NcoI*; in the normal, there is no cleavage, while in the patient (M.H.), the 43-bp fragment appears.

GENETICS AND PATHOGENESIS

MCAD deficiency is autosomal recessive [36–38]. The gene is on chromosome 1 and the nucleotide sequence of its cDNA has been established. A single mutation in which nucleotide 985 has been changed from A to G, leading to a lysine (K) to glutamic acid (E) change in residue 329 of the protein, accounts for virtually all of the patients identified prior to the addition of the disease to programs of newborn screening (Figures 38.7 and 38.8) [36–38]. Among 172 patients, 80.2 percent were homozygous for A \rightarrow G⁹⁸⁵ and 17.4 percent were heterozygous for this mutation [38]. Only 4 percent did not have this change on either allele. A second mutation was found in a patient with MCAD deficiency who was heterozygous for the A \rightarrow G⁹⁸⁵ and a 4-bp deletion [39]. Rapid screening is available for both mutations, which accounted for over 93 percent of all MCAD mutations in patients presenting with symptomatic disease. The common mutation leads to a high frequency of missplicing of mRNA [40], which would be expected to lead to variable phenotypic expression. Heterozygote detection and prenatal diagnosis can be carried out by testing for these mutations.

Identification of newborns in the United States with MCAD deficiency by MS/MS screening has yielded an incidence of one in 15,000 [41].

Mutation analysis revealed a lower incidence of the A \rightarrow G⁹⁸⁵ mutation among those identified by newborn screening than had been observed in populations diagnosed after the onset of clinical illness. A previously unrecognized mutation, T199C, which had never been found in patients with clinical illness, appears to code for a mild phenotype. Expression of the recombinant Y42H protein coded for by T199C yielded about 80 percent of control activity and even more with chaperonin coexpression, indicating that the mutation interferes with protein folding, but confers only mild interference with activity. The carrier frequency of this mutation appears to approximate one in 500. This mutation was never found in a sample of more than 90 patients identified by the presence of clinical illness. In this study, a mild acylcarnitine profile, as seen in patients heterozygous for A985G and T199C, had a C8 concentration of 0.5–2.0 $\mu\text{mol/L}$ and a C8/C10 ratio of 2–4, while the severe profile, found in all the A985G homozygotes studied, had a C8 over 2 and a ratio of over 4. These data confirm the reliability of newborn screening for clinical MCAD deficiency. Some 11 other mutations identified were rare, including IVS 8 + G \rightarrow T, which changed a splice consensus, were associated with severe deficiency of the enzyme.

Among infants identified by newborn screening, Zschocke *et al.* [43] found heterozygosity for the A985G mutation and Y67H in two patients, homozygosity for G267R in one, and S245L in two children of consanguineous parents. None of these patients had clinical disease up to six months of follow up at report. Urinary organic acids were

normal in these infants and their C8/C12 acylcarnitine ratios were lower than in patients with classic disease. Enzyme assay showed higher activity than in classic patients.

The enzyme, MCAD, is one of three mitochondrial acyl CoA dehydrogenases (Table 38.1) that catalyze the initial steps in the β -oxidation of fatty acids (Figure 38.1). Each is a flavin-containing dehydrogenase that is specific for CoA esters of specific chain length. MCAD accepts fatty acyl CoAs in which the acid chain length is 6–12 carbons in length. The enzyme may be assayed in leukocytes, liver, or cultured fibroblasts, as well as amniocytes. Immunochemical study of patients with the common mutation [44] revealed no evidence of cross-reacting material (CRM), and pulse chase labeling indicated that the enzyme is unstable.

The oxidation of fatty acids is not called upon in the production of energy until fasting has proceeded for some time. Glycogen stores suffice to provide carbohydrate for energy for 12 hours in most individuals. Thus, a history of hypoglycemia after a short fast implies a disorder of carbohydrate metabolism, while hypoglycemia after a prolonged fast implies a disorder of fatty acid oxidation. We have encountered exceptions to both rules, but in general when we have subjected patients in remission with disorders of fatty oxidation to fasting under controlled conditions, hypoglycemia has seldom ensued before 16–18 hours (Figure 38.5). This is consistent with the fact that the usual presentation is after seven months (median age, 13.5 months) [36], usually concomitant with a first infectious illness that leads to anorexia or prolonged vomiting and its attendant fasting. It also explains the fact that in some patients the first episode occurs in a teenager [14] or adult [42] (Figure 38.9). The recognition of asymptomatic affected adults is consistent with the fact that some people never experience a fast longer than 16 hours. We expect that the incidence of normality will be high in those infants detected through routine screening.

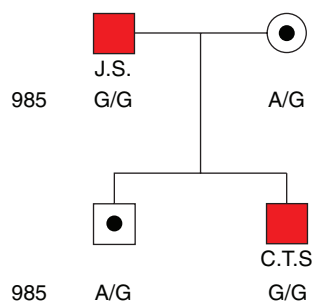


Figure 38.9 The pedigree of a family [42] in which a 12-month-old patient was admitted with hypoketotic hypoglycemia and the A→G⁹⁸⁵ mutation; he was found to have a father who was also homozygous for this mutation. The father had had two episodes of symptomatic hypoglycemia in infancy and had therefore studiously avoided fasting. The mother and a brother of the proband were heterozygous.

In our experience, the exceptional patient who developed hypoglycemia after shorter periods of fasting, was a young infant who developed multiple episodes during infancy. The occurrence of SIDS is of course another exception to the expected course [45–47], and fatal neonatal presentation has been reported [6] in an infant with hypoglycemia and normal levels of free carnitine who had severe lipid cardiomyopathy at autopsy. GCMS of the liver in patients with SIDS has yielded cis-4-decenoic acid (C10:1) in each of four infants found to have had MCAD deficiency [46]. The prognosis for survival appears to be particularly bad for those with a neonatal presentation, although overall mortality in the first episode may be as high as 60 percent [45]. In patients surviving to diagnosis, the prognosis is good. Physical and intellectual development may be normal, although abnormal psychometric tests of development were surprisingly frequent in the survivors reported by Iafolla *et al.* [19, 20]. We expect that the incidence of normality will be high in those infants detected through routine screening.

TREATMENT

The hallmark of treatment is the avoidance of fasting. Supplies of readily accepted and tolerated oral carbohydrate should be plentiful and accessible in the home. In a fragile infant, a supply of glucose (Monogel®), or even parenteral glucagon, may be useful. In the presence of vomiting or anorexia that prevents oral intake, parenteral glucose is mandatory. Admission to hospital is prudent, but sometimes remission can be accomplished in the emergency room. Rates of administration and concentrations should be adequate to reverse hypoglycemia and maintain normoglycemia. It is not sufficient to start 5 percent glucose and relax; we have seen patients in whom symptomatic hypoglycemia developed under those circumstances. In long-term management, we have routinely employed supplemental cornstarch, at least for evening and night feedings. The initial dosage we use is 0.5 g/kg (1 Tbsp = 8 g), usually working up to 1.0 g/kg. In the fragile infant referred to above, 2.0 g/kg appeared to be helpful. Some reduction in the intake of fat appears prudent, but this does not need to be excessive.

Supplementation with carnitine is currently controversial, but why this is so appears difficult to understand. Patients are demonstrably deficient in free carnitine in virtually any circumstance in which they have lived undiagnosed past early infancy [48]. The very high urinary esterified carnitine and its major increase with the specific increase in excretion of octanoylcarnitine and hexanoylcarnitine implies a detoxification function that should well be employed. During illness, octanoylcarnitine excretion increases dramatically when the patient is given intravenous carnitine [49]. Figure 38.5 illustrates considerably improved ketogenesis and an absence of symptoms despite fasting hypoglycemia in a patient treated

for only a few days with carnitine. An absence of effect after three months of treatment with carnitine in a five-month-old infant was reported because of the development of symptoms and hypoglycemia after 16.5 hours of controlled fasting [47]. However, the investigators permitted this patient to fast only 12 hours prior to treatment, which did restore concentrations of carnitine in plasma to normal and markedly increased urinary carnitine excretion. Furthermore, the blood level of 3-hydroxybutyrate rose to 0.84 mmol/L, while prior to carnitine it failed to exceed 0.38 mmol/L. In a study of five symptom-free patients [50], acylglycine excretion exceeded acylcarnitine excretion by a factor of 70 to 1, but the amounts could not be increased by supplemental oral glycine. Supplemental carnitine increased acylcarnitine excretion six-fold and caused a 60 percent reduction in acylglycine excretion. In another study of monitored fasting [51], a patient tolerated a 12-hour fast after treatment, whereas before, 12 hours of fasting had induced a depressed sensorium and acidosis, as well as the expected accumulation of free fatty acids in the blood and dicarboxylic acids in the urine. We suspect that the failure to recognize a role for carnitine in treatment stems from the fact that once diagnosed, these patients do so well if they avoid fasting. Most of our patients have not had a second episode requiring admission to hospital, but of course all of them have been treated with carnitine. An initial dose of 60–100 mg/kg is useful. During acute illness, we use 200–300 mg/kg intravenously. Treatment with 50–150 mg riboflavin/day was reported [52] to increase the activity of MCAD in lymphocytes of five patients with MCAD deficiency. Increases were very small in four, but a major increase in one patient, who began with 19 percent of control activity, suggests that supplementation may be a useful adjunct.

REFERENCES

1. Rhead WJ, Amendt BA, Fritchman KS, Felts SJ. Dicarboxylic aciduria: deficient 1-(14) C-octanoate oxidation and medium-chain acyl-CoA dehydrogenase in fibroblasts. *Science* 1983; **221**: 73.
2. Stanley CA, Hale DE, Coates PM *et al.* Medium-chain acyl-CoA dehydrogenase deficiency in children with non-ketotic hypoglycemia and low carnitine levels. *Pediatr Res* 1983; **17**: 877.
3. Nyhan WL. Abnormalities of fatty acid oxidation. *N Engl J Med* 1988; **319**: 1344.
4. Surtee R, Leonard JV. Acute metabolic encephalopathy: a review of causes, mechanisms and treatment. *J Inher Metab Dis* 1989; **12**(Suppl.): 42.
5. Rowe PC, Valle D, Brusilow SW. Inborn errors of metabolism in children referred with Reye's syndrome. *J Am Med Assoc* 1988; **260**: 3167.
6. Leung KC, Hammond JW, Chabra S *et al.* A fatal neonatal case of medium-chain acyl-coenzyme A dehydrogenase deficiency with homozygous A→G985 transition. *J Pediatr* 1992; **121**: 965.
7. Emery JL, Variend S, Howat AJ, Vawter GF. Investigation of inborn errors of metabolism in unexpected infant deaths. *Lancet* 1988; **2**: 29.
8. Harpey JP, Charpentier C, Paturneau-Jouas M. Sudden infant death syndrome and inherited disorders of fatty acid β -oxidation. *Biol Neonate* 1990; **58**(Suppl. 1): 70.
9. Miller ME, Brooks JG, Forbes N, Insel R. Frequency of medium-chain acyl-CoA dehydrogenase deficiency G-985 mutation in sudden infant death syndrome. *Pediatr Res* 1992; **31**: 305.
10. Matsubara Y, Kraus JP, Yang-Feng TL *et al.* Molecular cloning of cDNAs encoding rat and human medium-chain acyl-CoA dehydrogenase and assignment of the gene to human chromosome 1. *Proc Natl Acad Sci USA* 1986; **83**: 6543.
11. Coates PM, Hale DE, Stanley CA *et al.* Genetic deficiency of medium-chain acyl coenzyme A dehydrogenase: studies in cultured skin fibroblasts and peripheral mononuclear leukocytes. *Pediatr Res* 1985; **19**: 672.
12. Patel JS, Leonard JV. Ketonuria and medium-chain acyl-CoA dehydrogenase deficiency. *J Inher Metab Dis* 1995; **18**: 98.
13. Treem WR, Witzleben CA, Piccoli DA *et al.* Medium-chain and long-chain acyl-CoA dehydrogenase deficiencies: clinical, pathologic and ultrastructural differentiation from Reye's syndrome. *Hepatology* 1986; **6**: 1270.
14. Marsden D, Sege-Petersen K, Nyhan WL *et al.* An unusual presentation of medium-chain acyl coenzyme A dehydrogenase deficiency. *Am J Dis Child* 1992; **146**: 1459.
15. Stanley CA, Coates PM. Inherited defects of fatty acid oxidation which resemble Reye's syndrome: IV. Reye's syndrome. *J Natl Reye's Synd Found* 1985; **5**: 190.
16. Green A, Hall SM. Investigation of metabolic disorders resembling Reye's syndrome. *Arch Dis Child* 1992; **67**: 1313.
17. Saudubray JM, Martin D, De Lonlay P *et al.* Recognition and management of fatty acid oxidation defects: a series of 107 patients. *J Inher Metab Dis* 1999; **22**: 488.
18. Rice G, Brazelton T, Maginot K *et al.* Medium chain acyl-coenzyme A dehydrogenase deficiency in a neonate. *N Engl J Med* 2007; **357**: 17; Hoffman G, Rice G, Wilcken B. More on medium-chain acyl coenzyme A dehydrogenase deficiency in a neonate. *N Engl J Med* 2008; **358**.
19. lafolla AK, Thompson RJ Jr, Roe CR. Medium-chain acyl-coenzyme A dehydrogenase deficiency: clinical course in 120 affected children. *J Pediatr* 1994; **124**: 409.
20. lafolla AK, Millington DS, Chen YT *et al.* Natural course of medium chain acyl-CoA dehydrogenase deficiency. *Am J Hum Genet* 1991; **49**(Suppl.): 99.
21. Marsden D, Nyhan WL, Barshop BA. Creatine kinase and uric acid: early warning for metabolic imbalance resulting from disorders of fatty acid oxidation. *Arch Neurol* 2000; **160**: 599.
22. Davidson-Mundt A, Luder AS, Greene CL. Hyperuricemia in medium-chain acyl-coenzyme A dehydrogenase deficiency. *J Pediatr* 1992; **120**: 444.
23. Reinehr T, Burk G, Dietz B *et al.* Hyperuricemia as the main symptom of medium-chain acyl-CoA dehydrogenase deficiency. *Klin Padiatr* 1997; **209**: 357.
24. Ceyham M, Kanra G, Yilmaz Y *et al.* Rabies (diagnosis and discussion). *Am J Dis Child* 1992; **146**: 1215.

25. Bennett MJ, Rinaldo P, Millington DS *et al.* Medium chain acyl CoA dehydrogenase deficiency: postmortem diagnosis in a case of sudden infant death and neonatal diagnosis of an affected sibling. *Pediatr Pathol* 1991; **11**: 889.
26. Rinaldo P, O'Shea JJ, Coates PM *et al.* Medium chain acyl-CoA dehydrogenase deficiency: diagnosis by stable-isotope dilution measurement of urinary N-hexanoylglycine and 3-phenylpropionylglycine. *N Engl J Med* 1988; **319**: 1308.
27. Wanders RJA, Vreken P, Den Boer MEJ *et al.* Disorders of mitochondrial fatty acyl-CoA β -oxidation. *J Inherit Metab Dis* 1999; **22**: 442.
28. Bhala A, Bennett MJ, McGowan KL, Hale DE. Medium-chain acyl-coenzyme A dehydrogenase deficiency. *Pediatrics* 1993; **122**: 100.
29. Vreken P, van Lint AEM, Bootsma AH *et al.* Rapid diagnosis of organic acidemias and fatty acid oxidation defects by quantitative electrospray tandem-MS acyl-carnitine analysis in plasma. In: Quant PA, Eaton S (eds). *Current Views of Fatty Acid Oxidation and Ketogenesis: From Organelles to Point Mutations*. New York: Plenum Publishers, Kluwer Academic, 1999: 327–37.
30. Roe CR, Millington DAM, Bohan TP *et al.* Diagnostic and therapeutic implications of medium-chain acylcarnitines in the medium-chain acyl-CoA dehydrogenase deficiency. *Pediatr Res* 1985; **19**: 459.
31. Roe CR, Millington DS, Maltby DA, Kinnebrew P. Recognition of medium-chain acyl-CoA dehydrogenase deficiency in asymptomatic siblings of children dying of sudden infant death or Reye-like syndromes. *J Pediatr* 1986; **108**: 13.
32. Chace DH, Hillman SL, Van Hover JL, Naylor EW. Rapid diagnosis of MCAD deficiency: quantitative analysis of octanoylcarnitine and other acylcarnitines in newborn blood spots by tandem mass spectrometry. *Clin Chem* 1997; **43**: 2106.
33. Clayton PT, Doig M, Ghafari S *et al.* Screening for medium chain acyl-CoA dehydrogenase deficiency using electrospray ionisation tandem mass spectrometry. *Arch Dis Child* 1998; **79**: 109.
34. Bove KE. Letter to the editor. *Pediatr Pathol* 1992; **12**: 621.
35. Duran M, Hofkamp M, Rhead W. Sudden child death and 'healthy' affected family members with medium-chain acyl-Coenzyme A dehydrogenase deficiency. *Pediatrics* 1986; **78**: 1052.
36. Coates PM, Hale DE, Stanley CA *et al.* Genetic deficiency of medium-chain acyl-coenzyme A dehydrogenase: studies in cultured skin fibroblasts and peripheral mononuclear leukocytes. *Pediatr Res* 1985; **19**: 671.
37. Coates PM, Tanaka E (eds). Workshop on Molecular Aspects of MCAD deficiency: Mutations causing medium-chain acyl-CoA dehydrogenase deficiency: a collaborative compilation of the data from 172 patients. In: *New Developments in Fatty Acid Oxidation*. New York: Wiley-Liss, 1992: 499.
38. Matsubara Y, Narisawa K, Miyabayashi S *et al.* Identification of a common mutation in patients with medium-chain acyl-CoA dehydrogenase deficiency. *Biochem Biophys Res Commun* 1990; **171**: 498.
39. Ding JH, Yang BZ, Bao Y *et al.* Identification of a new mutation in medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. *Am J Hum Genet* 1992; **50**: 229.
40. Zhang Z, Zhou Y, Kelly DP *et al.* Delineation of RFLPs and multiple ALU sequences associated with the A985G mutation in human medium chain acyl CoA dehydrogenase. *Pediatr Res* 1992; **31**: 137A.
41. Andresen BS, Dobrowolski SF, O'Reilly L *et al.* Medium-chain acyl-CoA dehydrogenase (MCAD) mutations identified by MS/MS-based prospective screening of newborns differ from those observed in patients with clinical symptoms: identification and characterization of a new, prevalent mutation that results in mild MCAD deficiency. *Am J Hum Genet* 2001; **68**: 1408.
42. Bodman M, Smith D, Nyhan WL, Naviaux RK. Medium-chain acyl CoA dehydrogenase deficiency. *Arch Neurol* 2001; **58**: 811.
43. Zschocke J, Schulze A, Lindner M *et al.* Molecular and functional characterization of mild MCAD deficiency. *Hum Genet* 2001; **108**: 404.
44. Coates PM, Indo Y, Young D *et al.* Immunochemical characterization of variant medium-chain acyl-CoA dehydrogenase in fibroblasts from patients with medium-chain acyl-CoA dehydrogenase deficiency. *Pediatr Res* 1992; **31**: 34.
45. Touma EH, Charpentier C. Medium chain acylCoA dehydrogenase deficiency. *Arch Dis Child* 1992; **67**: 142.
46. Boles RG, Martin SK, Blitzer M, Rinaldo P. Biochemical diagnosis of fatty acid oxidation disorders by GC/MS analysis of post-mortem liver. *Pediatr Res* 1993; **33**: 126A.
47. Treem WR, Stanley CA, Goodman SI. Medium-chain acyl-CoA dehydrogenase deficiency: metabolic effects and therapeutic efficacy of long term L-carnitine supplementation. *J Inherit Metab Dis* 1989; **12**: 122.
48. Gillingham MB, Van Calcar SC, Ney DM *et al.* Nutrition support of long chain 3-hydroxyacyl CoA dehydrogenase deficiency – a case report and survey. *J Inherit Metab Dis* 1999; **22**: 123.
49. Roe CR, Millington DS, Kahler SG *et al.* Carnitine homeostasis in the organic acidurias. In: Tanaka K, Coates PM (eds). *Fatty Acid Oxidation: Biochemical and Molecular Aspects*. New York: Alan R Liss, 1990: 383–402.
50. Rinaldo P, Schmidt-Sommerfeld E, Posca AP *et al.* Effect of treatment with glycine and L-carnitine in mediumchain acyl-coenzyme A dehydrogenase deficiency. *J Pediatr* 1993; **122**: 580.
51. Waber L, Francomano C, Brusilow S *et al.* Medium chain acyl CoA dehydrogenase (MCD) deficiency. *Pediatr Res* 1984; **18**: 302A.
52. Duran M, Cleutjens BJM, Ketting D *et al.* Diagnosis of medium-chain acyl-CoA dehydrogenase deficiency in lymphocytes and liver by a gas chromatographic method: the effect of oral riboflavin supplementation. *Pediatr Res* 1991; **31**: 39.

Very long chain acyl CoA dehydrogenase deficiency

Introduction	289	Treatment	292
Clinical abnormalities	289	References	292
Genetics and pathogenesis	291		

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia, hepatomegaly, cardiomyopathy and myopathy, rhabdomyolysis, elevated creatinine kinase, lipid infiltration of liver and muscle, and defective activity of very long chain acyl CoA dehydrogenase (VLCAD).

INTRODUCTION

Very long chain acyl CoA dehydrogenase (VLCAD) is bound to the inner mitochondrial membrane. It was first delineated in 1992 [1] as catalyzing the dehydrogenation of acyl CoA esters of 14 to 20 carbon length in the first step of mitochondrial fatty acid oxidation (Figure 39.1). Within a year, there were three reports [2–5] of patients with deficiency of VLCAD, including some who had been previously reported as having long chain acyl CoA dehydrogenase (LCAD) deficiency [6]. It is now recognized that most such patients have VLCAD deficiency, and the LCAD enzyme catalyzes the specific oxidation of branched long chain acyl CoAs. The usual assay with palmitoyl CoA as substrate in the presence of electron transfer flavoprotein (ETF) would register deficiency of activity if either LCAD or VLCAD was deficient. The distinction can be made by immunochemical analysis.

VLCAD deficiency is relatively common. There were 12 patients among the series of 107 disorders of fatty acid oxidation in the Paris experience of Saudubray *et al.* [7]. Vianey-Saban and colleagues [8] reported 30 patients from

Lyon. The VLCAD gene has been isolated [9] and found to contain 20 exons; it is situated on chromosome 17p13 [10–12]. A small number of mutations has been identified [13–16]. VLCAD deficiency is the most common disorder of fatty acid oxidation in the Saudi population.

CLINICAL ABNORMALITIES

This disease may present in the first days of life. One patient [2] had metabolic acidosis at 2 days of age. The blood level of creatinine kinase (CK) was 3684 U/L and he had impressive dicarboxylic aciduria. A sibling died suddenly without cardiac abnormalities and had massive fatty infiltration in the liver. In six families of 11 patients with VLCAD deficiency, there were eight instances of sudden infant death syndrome (SIDS) or unexplained death [5]. Six of 11 patients in this series died between three and 14 months [5].

Hypertrophic cardiomyopathy was found in five of the six who died. This is characteristic of the severe phenotype. Nevertheless, diagnosis and treatment are consistent with

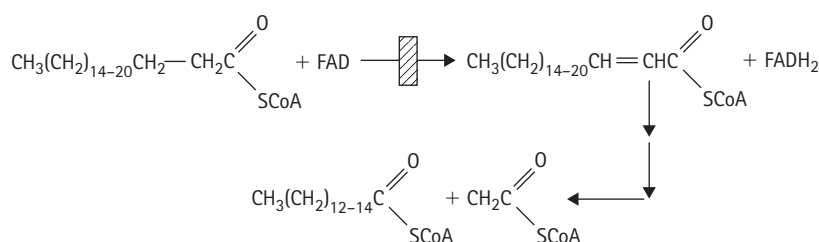


Figure 39.1 The very long chain acyl CoA dehydrogenase reaction. Following the formation of the enol product, the three successive reactions catalyzed by enoyl CoA hydratase, 3-hydroxyacyl CoA dehydrogenase, and 3-ketoacyl CoA hydratase yield ultimately acetyl CoA unless the deletion puts it back together and a fatty acyl CoA of two less carbons.



Figure 39.2 DS: A 16-month-old girl with the very long chain acyl CoA dehydrogenase deficiency. At 12 months of age, she was admitted in shock with a blood sugar of 0 and a seizure. She had modest dicarboxylic aciduria. Tandem mass spectrometry revealed elevated long chain acyl carnitine esters. She was thought to have hypopituitarism and hypothyroidism on the basis of abnormal test results at 12 months, but by six years these abnormalities disappeared, and she has grown well without replacement therapy since that time.



Figure 39.3 AG: A two-year-old girl with very long chain acyl CoA dehydrogenase deficiency. Diagnosis was made at 2 days of age; a previous sibling had died of a disorder of fatty acid oxidation. Acylcarnitine profile revealed elevated C14:1, C16, and C18:1. She had bilateral inverted nipples.

survival, reversal of cardiomyopathy, and relative health at time of report [4, 5, 17]. One patient who had hypoketotic hypoglycemia and cardiomyopathy died at 8 days of age of a penetrating duodenal ulcer and peritonitis [18].

The disease has been divided into two forms: the early severe form with cardiac involvement [19, 20] and the milder form with hypoglycemia as in medium chain acyl CoA dehydrogenase (MCAD) deficiency, but both may have hypoketotic hypoglycemia (Figures 39.2 and 39.3), and there really is a third phenotype characterized by episodic rhabdomyolysis and myoglobinuria [21]. There is some merging of these clinical forms, but distinction may be useful because it tends to correlate with amounts of residual enzyme activity.

Neonatal presentations include lethargy, tachypnea, or seizures, and hypoglycemia; metabolic acidosis or arrhythmia may be found. This is followed by decompensation and evidence of hypertrophic cardiomyopathy. There may be pericardial effusion. Approximately 50 percent of patients have died within two months of initial symptomatology [17, 22]. Of 54 patients, 25 had the severe form, 75 percent with onset in the first 3 days of life, of whom 92 percent had cardiomyopathy [19].

Some patients have had a more typical fatty acid oxidation presentation with fasting intolerance and acute hypoketotic hypoglycemia, usually presenting at the first intercurrent infection, and followed by episodic hypoglycemia. Patients or previous siblings have been diagnosed as having Reye syndrome [23]. Plasma ammonia may be elevated. Uric acid and CK are also high during attacks. An interesting variation on this theme was a patient who presented at two years with hypoglycemia and encephalopathy (glucose 1.7 mmol/L) and acidosis resulting from massive ketosis [24]. Because of this, a disorder of fatty acid oxidation was not considered, although the CK was 5373 U/L. The diagnosis was made when the acylcarnitine profile revealed elevated tetradecanoylcarnitine and was confirmed by enzyme analysis.

A third presentation is reminiscent of that of carnitine palmitoyl transferase (CPT) II with episodic muscle pains, rhabdomyolysis, and myoglobinuria [25, 26]. One 28-year-old woman experienced her first symptoms, which were induced by exercise, at 19 years [26]. Levels of CK in the blood were very high.

Plasma free-carnitine may be normal or low. Urinary carnitine may be low, especially at times of acute illness, as long chain acylcarnitine esters are not well excreted by the kidney. The accumulation in plasma of C14:1 carnitine esters is an important marker on acylcarnitine profile (Figure 39.4), and may be detected in blood spots in newborn screening [27]. Levels of C14:1, C14, C16, and C18:1 may be elevated, and the ratios of C14:1 to C14 or C14:1 to C18:1 may be particularly useful in diagnosis [23, 28]. Incubation of fibroblasts with ^{13}C -labeled palmitate revealed accumulation in C16; in this system C14:1 was not elevated [29].

Organic acid analysis of the urine reveals dicarboxylic

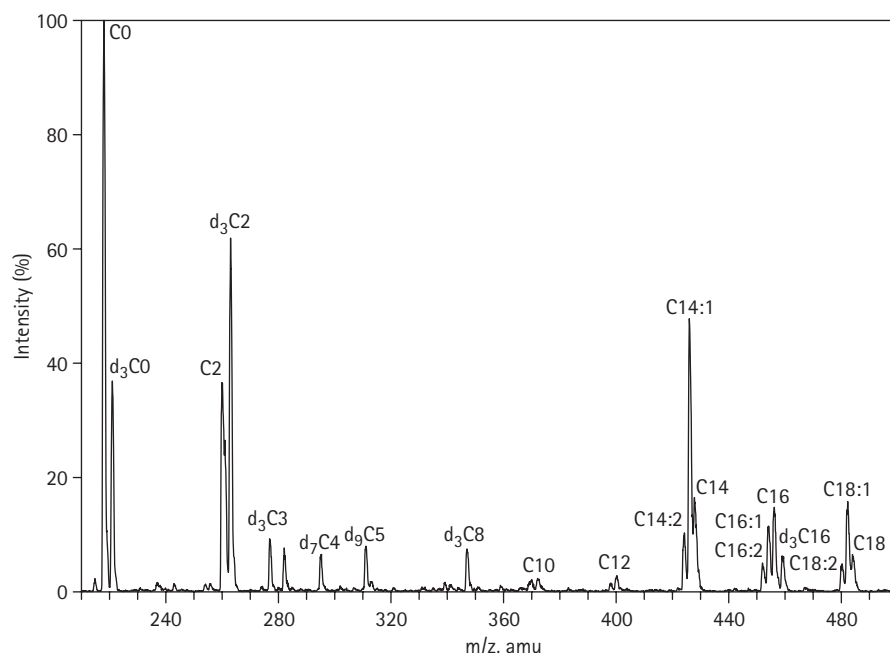


Figure 39.4 Acylcarnitine profile of the blood plasma of a patient with very long chain acyl CoA dehydrogenase deficiency. The patient was being treated with carnitine, and the C0 was elevated. The key compound was C14:1 acylcarnitine, but all of the long chain acylcarnitine esters were elevated. Illustration provided by Jon Gangoiti of UCSF.

aciduria. Unfortunately, during interepisode periods of health, when many diagnostic work ups occur, organic acid analysis is normal. During episodes, there is medium chain, as well as long chain dicarboxylic aciduria indicating the functioning of peroxisomal β - and ω -oxidation.

Pathologic examination reveals hepatic steatosis and deposits of lipid in cardiac and skeletal muscle. Mitochondrial appearance may be abnormal [30]. Peroxisomes may be enlarged.

Newborn screening has indicated that VLCAD deficiency is the most common of the defects in long chain fatty acid oxidation with a prevalence of 1:50,000 to 1:120,000 [31]. A level $>1 \mu\text{mol/L}$ correlates very well with enzyme- or mutation-proven disease. However, cut-off levels of $<0.25 \mu\text{mol/L}$ yield a number of false positives. Thus, confirmatory assays are necessary. A repeat screen or a plasma acylcarnitine profile is not that useful, because a normal value may be found in patients with the disease. Oxidation of palmitoyl CoA in lymphocytes with high performance liquid chromatography (HPLC) separation of the products, particularly 2-hexadecenoyl CoA [31, 32], has been useful in making a definitive diagnosis. Mutational analysis has become a common approach to this problem, but finding a single mutation does not prove heterozygosity because this has been found in the enzyme-proven patient [31].

GENETICS AND PATHOGENESIS

VLCAD deficiency is transmitted in an autosomal recessive fashion. The enzyme was purified from rat liver mitochondria [1]. It requires ETF as the electron receptor. Unlike the other mitochondrial acyl CoA dehydrogenases,

it is a heterodimer of 71 kDa. It does not crossreact with antibodies against LCAD or other acyl CoA dehydrogenases. Its activity is greatest against C16, palmitoyl CoA, and activity is ten times that of LCAD. Deficiency of VLCAD may be demonstrated in cultured fibroblasts. Antibody against VLCAD is reduced by 66–75 percent, and this may be demonstrated by Western blot analysis.

When antibody to LCAD became available, nine cell lines previously thought to be deficient in LCAD were tested and found to have normal immunoreactive LCAD protein [6], and testing via immunoblot analysis against VLCAD revealed them all to be VLCAD-deficient. Low VLCAD activity was also demonstrated by testing for enzyme activity in the presence of anti-LCAD antibody which did not alter activity [3].

Rapid indication of the diagnosis has been reported by the study of β -oxidation and VLCAD in lymphocytes [31] and the method has been adapted for prenatal diagnosis of chorionic villus material. Prenatal diagnosis has also been made by assay of the dehydrogenation of palmitoyl CoA in amniocytes [33].

In vitro studies of the incubation of fibroblasts with deuterium-labeled palmitate and carnitine followed by assay of the pattern of enrichment of acylcarnitines [34] has been reported to correlate well with the different phenotypes of severe cardiomyopathy and those without cardiomyopathy. The ratio of deuterated C16 to deuterated C12 has been discriminatory. Identification of 13 patients with myopathic phenotypes was made by immunoblot chemical of biopsied muscle [35].

The gene for VLCAD has been cloned and sequenced [36]. A number of mutations has been identified in study of 32 unrelated patients [19]. In general, patients with the more severe phenotype had alleles coding for truncated

proteins or proteins lacking amino acids consistent with predicted null activity [18, 19, 22, 25], whereas those with the milder phenotypes had alleles with missense mutations [19]. In a 14-year-old patient with recurrent myalgia and CK elevation with moderate exercise, the attenuated phenotype was associated with mutations A416T and R450H each of which expressed as temperature-sensitive enzymes [36]. The woman whose symptoms began at 19 years, had two missense mutations (G145C/R375W) [25]. An interesting S583W mutation demonstrated that association of the mature VLCAD protein with the inner mitochondrial membrane is required for activity, because the protein is imported normally [15]. Of 37 patients, only one mutation was found in seven [37], despite sequencing of all of the exons. A high incidence of cardiomyopathy provides caution about concluding that the finding of only one mutation indicates heterozygosity.

TREATMENT

Treatment aimed at the hypoglycemia emphasizes the avoidance of fasting and prompt intervention with parenteral glucose-containing solutions when fasting is unavoidable. We provide parents with a letter, which can be produced at a medical facility, stressing the necessity of intravenous glucose under such conditions, even if an initial glucose level is normal. Fasting attendant upon surgery and anesthesia, particularly for minor procedures may be particularly dangerous [38]. Early preparation with intravenous glucose prevents problems. Cornstarch supplementation may be useful (2 g/kg at bedtime). Medium chain triglyceride (MCT) appears to be therapeutic and surviving patients have done well after initiation of MCT supplementation. A diet low in fat (5–10 percent of calories in long chain triglycerides) supplemented with MCT (30 percent of total calories or 85–90 percent of the calories from fat) was credited with reversal of hypertrophic cardiomyopathy [18]; the patient also received 50–100 g/kg of carnitine. Similar resolution of cardiomyopathy was reported in a boy treated with a similar regimen; he and his prenatally diagnosed sister, had normal cardiac and developmental function at follow up [30]. In the course of treatment, deficiency of ω -6 fatty acids, such as DHA and arachidonic acids, has been reported [39], but neither patient had any symptoms of deficiency; specifically there was no pigmentary retinopathy as in LCHAD deficiency (Chapter 40), and the levels do not seem especially low to those of us monitoring very low fat diets, such as those employed for lipoprotein lipase deficiency (Chapter 87). Carnitine administration, at least to restore levels of free-carnitine, appears to be indicated.

Carnitine therapy has become controversial in this as well as other long chain fatty acid oxidation disorders, largely on the basis of anecdote. Too, long chain acylcarnitine esters have been reported to promote ischemic damage or abnormal post-ischemic function in

experimental animals [40, 41], and their prevention by inhibitors of acylcarnitine formation, but another study found no effect of a CPT I inhibitor and obtained evidence that the ischemic damage resulted from the fatty acids [42]. In VLCAD-deficient mice, carnitine supplementation increased the content of acylcarnitine esters in muscle and liver without replenishing free carnitine [43]. Incubation of hepatic cells with carnitine decreased their viability. Treatment with carnitine was reported to ameliorate recurrent myoglobinuria in an 11-year-old patient with VLCAD deficiency [44], but to be without effect in another patient [25].

In fibroblasts of patients with CPT II deficiency, pharmacologic enhancement of enzyme activity *in vitro* has been demonstrated with benzaifibrate, an agonist of the peroxisome proliferator activated receptors (PPARs) [45]. This has been extrapolated to VLCAD fibroblasts with similar enhancement of oxidation capacity *in vitro* [46, 47].

REFERENCES

1. Izai K, Uchida Y, Orii T *et al.* Novel fatty acid β -oxidation enzymes in rat liver mitochondria. I. Purification and properties of very-long-chain acyl-coenzyme A dehydrogenase. *J Biol Chem* 1992; **267**: 1027.
2. Bertrand C, Largilliere C, Zabot MT *et al.* Very long chain acyl-CoA dehydrogenase deficiency: identification of a new inborn error of mitochondrial fatty acid oxidation in fibroblasts. *Biochim Biophys Acta* 1993; **1180**: 327.
3. Aoyama T, Uchida Y, Kelley RI *et al.* A novel disease with deficiency of mitochondrial very-long-chain acyl-CoA dehydrogenase. *Biochim Biophys Res Commun* 1993; **191**: 1369.
4. Yamaguchi S, Indo Y, Coates PM *et al.* Identification in very-long-chain acyl-CoA dehydrogenase deficiency. *Pediatr Res* 1993; **34**: 111.
5. Vianey-Saban C, Divry P, Zabot MT, Mathieu M. Mitochondrial very long chain acyl-CoA dehydrogenase deficiency (VLCAD): identification of this new inborn error of fatty acid oxidation in 11 patients. Proceedings VI International Congress of Inborn Errors of Metabolism, 1994, Abstr. 88 Wp.1.
6. Indo Y, Coates PM, Hale DE, Tanaka K. Immunochemical characterization of variant long chain acyl-CoA dehydrogenase in cultured fibroblasts from nine patients with long chain acyl CoA dehydrogenase deficiency. *Pediatr Res* 1991; **30**: 211.
7. Saudubray JM, Martin D, De Lonlay P *et al.* Recognition and management of fatty acid oxidation defects: a series of 107 patients. *J Inher Metab Dis* 1999; **22**: 488.
8. Vianey-Saban C, Divry P, Brivet M *et al.* Mitochondrial very-long-chain acyl-coenzyme A dehydrogenase deficiency: clinical characteristics and diagnostic considerations in 30 patients. *Clin Chim Acta* 1998; **269**: 43.
9. Aoyama T, Ueno I, Kamijo T, Hashimoto T. Rat very-long-chain acyl-CoA dehydrogenase, a novel mitochondrial acyl-CoA dehydrogenase gene product, is a rate-limiting enzyme in a

- long-chain fatty acid β -oxidation system. cDNA and deduced amino acid sequence and distinct specificities of the cDNA-expressed protein. *J Biol Chem* 1994; **269**: 19088.
10. Strauss AW, Powell CK, Hale DE *et al*. Molecular basis of human mitochondrial very-long-chain acyl-CoA dehydrogenase deficiency causing cardiomyopathy and sudden death in childhood. *Proc Natl Acad Sci USA* 1995; **92**: 10496.
 11. Aoyama T, Souri M, Ueno I *et al*. Cloning of human very-long-chain acyl-coenzyme A dehydrogenase and molecular characterization of its deficiency in two patients. *Am J Hum Genet* 1995; **57**: 273.
 12. Orii K, Aoyama T, Souri M *et al*. Genomic DNA organization of human mitochondrial very-long-chain acyl-CoA dehydrogenase and mutation analysis. *Biochem Biophys Res Commun* 1995; **217**: 987.
 13. Aoyama T, Wakui K, Fukushima Y *et al*. Assignment of the human mitochondrial very-long-chain acyl-CoA dehydrogenase gene (LCACD) to 17p13 by *in situ* hybridization. *Genomics* 1996; **37**: 144.
 14. Souri M, Aoyama T, Orii K *et al*. Mutation analysis of very-long-chain acyl-coenzyme A dehydrogenase (VLCAD) deficiency: identification and characterization of mutant VLCAD cDNAs from four patients. *Am J Hum Genet* 1996; **58**: 97.
 15. Souri M, Aoyama T, Hoganson G, Hashimoto T. Very-long-chain acyl-CoA dehydrogenase subunit assembles to the dimmer form on mitochondrial inner membrane. *FEBS Lett* 1998; **426**: 187.
 16. Andresen BS, Bross P, Vianey-Saban C *et al*. Cloning and characterization of human very-long-chain acyl-CoA dehydrogenase cDNA, chromosomal assignment of the gene and identification in four patients of nine different mutations within the VLCAD gene. *Hum Mol Genet* 1996; **5**: 461.
 17. Cox GF, Souri M, Aoyama T *et al*. Reversal of severe hypertrophic cardiomyopathy and excellent neuropsychologic outcome in very-long-chain acyl-coenzyme A dehydrogenase deficiency. *J Pediatr* 1998; **133**: 247.
 18. Pust B, Berger A, Hennenberger A *et al*. Very-long-chain acyl-coenzyme A dehydrogenase deficiency (VLCADD) with gastrointestinal hemorrhage as a fatal complication. *J Inherit Metab Dis* 1996; **19**: P105 (Abstr.).
 19. Anderson BS, Olpin S, Poorthuis BJ *et al*. Clear correlation of genotype with disease phenotype in very-long-chain acyl-CoA dehydrogenase deficiency. *Am J Hum Genet* 1999; **64**: 479.
 20. Nada MA, Vianey-Saban C, Roe CR. Very-long-chain-acyl-CoA dehydrogenase deficiency: two phenotypes with distinctive biochemical findings. *J Inherit Metab Dis* 1996; **19**: P105 (Abstr.).
 21. Ogilvie I, Pourfarzam M, Jackson S *et al*. Very long-chain acyl coenzyme A dehydrogenase deficiency presenting with exercise-induced myoglobinuria. *Neurology* 1994; **44**: 467.
 22. Aoyama T, Souri M, Ushikubo S *et al*. Purification of human very-long-chain acyl-CoA dehydrogenase and characterization of its deficiency in seven patients. *J Clin Invest* 1995; **95**: 2465.
 23. Hahn S-H, Lee E-H, Jung J-W *et al*. Very long chain acyl coenzyme A dehydrogenase deficiency in a 5-month-old Korean boy: identification of a novel mutation. *J Pediatr* 1999; **135**: 250.
 24. Wraige E, Champion MP, Turner C, Dalton RN. Fat oxidation defect presenting with overwhelming ketonuria. *Arch Dis Child* 2002; **87**: 428.
 25. Ogilvie I, Pourfarzam M, Jackson S *et al*. Very long chain acyl coenzyme A dehydrogenase deficiency presenting with exercise-induced myoglobinuria. *Neurology* 1994; **44**: 467.
 26. Pou-Serradell A, Ribes A, Briones P *et al*. Myopathic presentation in an adult woman with a very long chain acyl-coenzyme A dehydrogenase deficiency. *J Inherit Metab Dis* 2001; **24**(Suppl. 1): 70.
 27. Wood JC, Magera MJ, Rinaldo P *et al*. Diagnosis of very long chain acyl-dehydrogenase deficiency from an infant's newborn screening card. *Pediatrics* 2001; **107**: 173.
 28. Onkenhout W, Venizelos V, van der Poel PF *et al*. Quantification of intermediates of unsaturated fatty acid metabolism in plasma of patients with fatty acid oxidation disorders. *Clin Chem* 1995; **41**: 1467.
 29. Tyni T, Pourfarzam M, Turnbull DM. Analysis of mitochondrial fatty acid oxidation intermediates by tandem mass spectrometry from intact mitochondria prepared from homogenates of cultured fibroblasts, skeletal muscle cells, and fresh muscle. *Pediatr Res* 2002; **52**: 64.
 30. Treem WR, Witzleben CA, Piccoli DA *et al*. Medium-chain and long-chain acyl-CoA dehydrogenase deficiency. Clinical, pathologic, and ultrastructural differentiation from Reye's syndrome. *Hepatology* 1986; **6**: 1270.
 31. Liebig M, Schymik I, Mueller M *et al*. Neonatal screening for very long-chain acyl-CoA dehydrogenase deficiency: enzymatic and molecular evaluation of neonates with elevated C14:1-carnitine levels. *Pediatrics* 2006; **3**: 1065.
 32. Tajima G, Sakura N, Shirao K *et al*. Development of a new enzymatic diagnosis method for very-long-chain acyl-CoA dehydrogenase deficiency by detecting 2-hexadecenoyl-CoA production and its application in tandem mass spectrometry-based selective screening and newborn screening in Japan. *Pediatr Res* 2008; **64**: 667.
 33. Sluysmans T, Tuerlinckx D, Hubinont C *et al*. Very long chain acyl-coenzyme A dehydrogenase deficiency in two siblings: evolution after prenatal diagnosis and prompt management. *J Pediatr* 1997; **131**: 44.
 34. Vianey-Saban C, Divry P, Brivet M *et al*. Mitochondrial very-long-chain acyl-coenzyme A dehydrogenase deficiency: clinical characteristics and diagnostic considerations in 30 patients. *Clin Chim Acta* 1998; **269**: 43.
 35. Ohashi Y, Hasegawa Y, Murayama K *et al*. A new diagnostic test for VLCAD deficiency using immunohistochemistry. *Neurology* 2004; **62**: 2209.
 36. Fukao T, Watanabe H, Orii KE *et al*. Myopathic form of very-long chain acyl-CoA dehydrogenase deficiency: evidence for temperature-sensitive mild mutations in both mutant alleles in a Japanese girl. *Pediatr Res* 2001; **49**: 227.
 37. Mathur A, Sims HF, Gopalakrishnan D *et al*. Molecular heterogeneity in very-long-chain acyl-CoA dehydrogenase deficiency causing pediatric cardiomyopathy and sudden death. *Circulation* 1999; **99**: 1337.

38. Roe CR, Wiltse HE, Sweetman L, Alvarado LL. Death caused by perioperative fasting and sedation in a child with unrecognized VLCAD deficiency. *J Pediatr* 2000; **136**: 397.
39. Ruiz-Sanz JI, Aldamiz-Echevarria L, Arrizabalaga J *et al*. Polyunsaturated fatty acid deficiency during dietary treatment of very long-chain acyl-CoA dehydrogenase deficiency. Rescue with soybean oil. *J Inherit Metab Dis* 2001; **24**: 493.
40. Corr PB, Creer MH, Yamada KA *et al*. Prophylaxis of early ventricular fibrillation by inhibition of acyl-carnitine accumulation. *J Clin Invest* 1989; **83**: 927.
41. Yamada KA, McHowat J, Yan G-X *et al*. Cellular uncoupling induced by accumulation of long-chain acylcarnitine during ischemia. *Circulation Res* 1994; **74**: 83.
42. Madden MC, Wolkowitz PE, Pohost GM *et al*. Acylcarnitine accumulation does not correlate with reperfusion recovery in palmitate-perfused rat hearts. *Am J Physiol* 1995; **268**: H2505.
43. Primassin S, Ter Veld F, Mayatepek E *et al*. Carnitine supplementation induces acylcarnitine production in tissue of very long-chain acyl-CoA dehydrogenase-deficient mice, without replenishing low free carnitine. *Pediatr Res* 2008; **63**: 632.
44. Straussberg R, Harel L, Varsano I *et al*. Recurrent myoglobinuria as a presenting manifestation of very long chain acyl coenzyme A dehydrogenase deficiency. *Pediatrics* 1997; **99**: 894.
45. Djouadi F, Bonnefont JP, Thuiller L *et al*. Correction of fatty acid oxidation in carnitine palmitoyltransferase II-deficient cultured skin fibroblasts by benzaifibrate. *J Clin Endocr Metab* 2003; **90**: 1791.
46. Gobin-Limballe S, Djouadi F, Aubey F *et al*. Genetic basis for correction of very-long-chain acylcoenzyme A dehydrogenase deficiency by benzaifibrate in fibroblasts: toward a genotype-based therapy. *Am J Hum Genet* 2007; **81**: 1133.
47. Djouadi F, Aubey F, Schlemmer D *et al*. Bezafibrate increases very-long-chain acyl-CoA dehydrogenase protein and mRNA expression in deficient fibroblasts and is a potential therapy for fatty acid oxidation disorders. *Hum Mol Genet* 2005; **14**: 2695.

Long chain L-3-hydroxyacyl CoA dehydrogenase – (trifunctional protein deficiency)

Introduction	295	Treatment	299
Clinical abnormalities	296	References	299
Genetics and pathogenesis	297		

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia, episodic rhabdomyolysis, hypotonia, cardiomyopathy, hepatic disease, peripheral neuropathy, pigmentary retinopathy, 3-hydroxydicarboxylic aciduria, carnitine deficiency and defective activity of the trifunctional protein or isolated deficiency of the long chain L-3-hydroxyacyl CoA dehydrogenase (LCHAD) subunit or the long chain ketothiolase (LCKAT) subunit. Maternal acute fatty liver of pregnancy during carriage of a fetus with LCHAD deficiency.

INTRODUCTION

Long chain L-3-hydroxyacyl CoA dehydrogenase deficiency was first reported [1, 2] in 1983 in a boy who had many attacks of hypoketotic hypoglycemia starting at nine months of age, had hypotonia and cardiomyopathy, and went on to develop massive hepatic necrosis, and died at 19 months. There was long chain acylcarnitine accumulation in plasma and 3-hydroxydicarboxylic aciduria. The activity of LCHAD was demonstrated to be defective in an assay in which 3-ketopalmitoyl CoA was the substrate.

The LCHAD enzyme is a component of the trifunctional protein (TFP) bound to the inner mitochondrial membrane [3, 4]. The protein is an octamer with two distinct α and β subunits coded for by the *HADHA* and *HADHB* genes. Its three activities are long chain 2-enoylCoA hydratase, LCHAD, and long chain 3-oxoacylCoA thiolase (LCKAT). Its LCHAD activity against 3-hydroxyacylCoA substrates is optimal for compounds of C12–C16 chain

length, in contrast to the short chain-3-hydroxyacylCoA dehydrogenase (SCHAD), where specificity is optimal at C6. LCHAD action is highest at C16 and inactive at C4. The thiolase and enoyl hydratase activities also have long chain specificities. The LCHAD enzyme catalyzes the reversible dehydration of the 3-hydroxy group to a 3-keto group, and nicotinamide adenine dinucleotide (NAD) is the hydrogen acceptor (Figure 40.1). Patients with LCHAD deficiency may be deficient in LCHAD activity specifically, or may be deficient in all three activities of the TFP. The genes for the α and β subunits have been cloned [5]. The α cDNA codes for an 82,598 Da precursor of a mature 78,969 Da protein. In mitochondrial trifunctional protein (MTP) deficiency, all three activities, dehydrogenase, hydratase, and this thiolase are deficient [6]. Mutational analysis has revealed a number of distinct mutations including one that appears to be common, a G1528C point mutation in the dehydrogenase coding region that changes a glutamic acid to a glutamine [6, 7]. Isolated deficiency of LCHAD

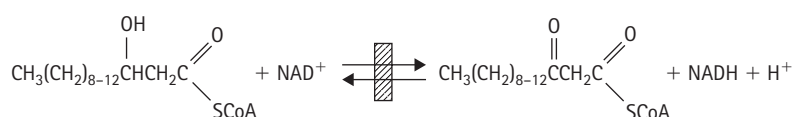


Figure 40.1 The reaction catalyzed by long chain L-3-hydroxyacyl CoA dehydrogenase (LCHAD). The product is then involved in the 3-ketothiolase reaction in which the bond is cleaved and acetyl CoA split off, yielding a fatty acid CoA ester of two less carbons.

is coded for by mutation in the *HADHA* gene [8]. Isolated deficiency of LCKAT activity is coded for by mutations in the *HADHB* gene [9]. Generalized TFP deficiency can also result from mutation in the *HADHB* gene.

CLINICAL ABNORMALITIES

Patients with LCHAD deficiency usually present in late infancy with the typical clinical picture of a disorder of fatty acid oxidation of which the hallmark feature is acute hypoketotic hypoglycemia (Figures 40.2 and 40.3). These episodes often begin late in the first year of life, with the first long fast, usually caused by an intercurrent infection



Figure 40.2 UJ: A nine-month-old boy with long chain L-3-hydroxyacyl CoA dehydrogenase deficiency. He had a number of episodes of hypoglycemia starting at five months of age, when he was found to have hepatomegaly and hepatic steatosis. Fasting and loading with long chain triglycerides led to hypoglycemia, while medium chain triglyceride (MCT) loading was uneventful. Later, he had an episode of myoglobinuria and massive elevation of creatinine phosphokinase.



Figure 40.3 The feeding tube reflected the need, prior to referral, for virtually continuous feeding to maintain normoglycemia.

illness and ushered in with vomiting [8, 10–13]. Many have been diagnosed as having Reye syndrome. Mean age of onset in 50 patients was between five and eight months. The disease may be a cause of sudden infant death, even neonatal [14, 15]. With prompt diagnosis and treatment, acute neonatal cardiorespiratory arrest may yield to resuscitation and a favorable prognosis [16]. During the acute episode, levels of creatine phosphokinase (CK) and uric acid are elevated [17].

The acute episode may begin with a seizure; the electroencephalogram (EEG) may be abnormal. Most patients are hypotonic at least in infancy. Patients may be difficult to feed and gavage feeding may be required [14]. Some may display failure to thrive.

Later episodes are often ushered in with pains in the legs. Rhabdomyolysis leads to myoglobinuria [18]. Patients may first present as adults with exercise-induced muscle pains and rhabdomyolysis. Levels of CK may be very high (15,000–165,000 IU). Examination may reveal profound weakness, little movement, and the assumption of a frog-leg position.

Some patients with myopathic presentations have had rapidly fatal cardiomyopathy in infancy [19, 20]. Acute life-threatening cardiac episodes may be followed by tetraparesis [20]. Such patients may, or may not have had earlier episodes of hypoketotic hypoglycemia. Examination of the heart may reveal cardiomegaly, poor heart sounds, and gallop rhythm. The electrocardiogram reveals sinus tachycardia, a long QT, ventricular tachycardia, or a long left ventricular hypertrophy [20]. Echocardiography may reveal dilatation and poor contractility [19]. Pericardial effusion has been reported, as well as tamponade [13, 20]. Others have had a more indolent, myopathic presentation in which ventricular hypertrophy is found on echocardiography or electrocardiography in the absence of symptoms [14].

Hepatic dysfunction is another characteristic of the disease [1, 2, 13, 14, 18]. Most patients have hepatomegaly [12, 19]. Some have had acute cholestatic jaundice as neonates, and this may be transient [12]. The other end of the scale is massive total hepatic necrosis in infancy [1]. Jaundice may develop in infancy along with elevation in the blood levels of transaminases. Ultrasound or other imaging may indicate fatty infiltration of the liver. Biopsy reveals accumulation of fat and fibrosis.

An unusual result of hepatic disease was cholestatic jaundice and impaired 25-hydroxylation of vitamin D leading to hypocalcemia and a presentation at two months of age with a tonic-clonic seizure [21].

A more unusual complication is the acute fatty liver of pregnancy in a mother carrying a fetus with LCHAD deficiency [22]. The heterozygous mother may have pre-eclampsia and urinary protein, or the hypertension, elevated liver enzymes and low platelets (HELLP) syndrome. As many as approximately 20 percent of pregnancies at risk may be complicated by one of these problems [8].

It has increasingly been recognized that pigmentary retinopathy is a potential complication of LCHAD deficiency [10, 11, 23–26]. This may occur in as many as 70 percent of patients, but as yet the true incidence is unclear, as visual problems are progressive, and few patients have been followed for very long. In a series of 28 children, a pattern of ophthalmologic progression emerged [23]. Of 15 patients who died at ages from three to 14 months, vision had been normal for age. In the oldest survivors (16 and 31 years), visual loss was progressive. In 11 children, granular retinal pigment was seen at four months to five years. The two long-term patients had progressive atrophy of the choroid and retina, axial myopia, and scotomata. All four longer-surviving patients had lenticular opacities. The electroretinogram deteriorated during the final decade and became unrecordable in the oldest patient. Posterior staphylomas were seen in the two oldest patients.

Another clinical abnormality unusual in disorders of fatty acid oxidation that has been observed with time in LCHAD deficiency is peripheral neuropathy [18, 23, 24]. By adolescence, neuropathy and retinopathy may be the major clinical problems [23]. Deep tendon reflexes may be absent even in infancy [25]. The patient may toe-walk and display an equinus deformity. Extensor plantar responses have been reported [25]. In one patient, mild peripheral neuropathy of adult onset was the only clinical abnormality [27]. Intelligence in these patients has usually been normal, but of course prolonged hypoglycemia always carries a risk of injury to the central nervous system, and a number of patients have had impaired mental development and/or had a seizure disorder. Mortality has been as high as 38 percent [8]. Morbidity in surviving patients has also been high [8], especially acute muscle problems and episodic metabolic derangement [8]. On the other hand, it was notable that all who died did so within three months of diagnosis. In those surviving, none had cardiomyopathy, and their clinical condition was good despite recurrent muscle problems or diminished visual acuity.

Clinical manifestations of MTP deficiency and isolated LCHAD deficiency are generally indistinguishable, but in one series [12], 42 percent of patients with MTP had rapidly progressive deterioration; eight of the nine died of cardiac disease within two months. The ninth died of hepatic failure at 4 weeks. Two patients diagnosed prenatally died despite treatment; one had hydrops fetalis. Two pregnancies were complicated by HELLP syndrome. Among five Japanese patients with MTP deficiency [28], two had onset within the first days of life with lactic acidemia, hypoglycemia, and hyperammonemia. They died shortly of cardiac arrest. Two had hepatic presentations and rhabdomyolysis in late infancy. Another had an onset at 15 years of muscle pain, weakness, and rhabdomyolysis. Isolated LKAT deficiency was described in a newborn with lactic acidemia, pulmonary edema, and cardiomyopathy. He developed acute heart failure and died at 6 weeks of age [9].

The clinical chemistry in the acute illness may reveal hyperammonemia (68–400 mmol/L). This, with the hypo-

glycemia, hepatomegaly and elevation of transaminases is what has led to a diagnosis of Reye syndrome. The CK is elevated and so is the level of uric acid [17]. Lactic acidemia may accompany the acute episode, or there may be persistent lactic acidemia [1, 14, 18, 25]. Fatal neonatal lactic acidemia has been reported [28]. Carnitine is low, especially free carnitine. Free fatty acids are increased, and the ratio of free fatty acids to 3-hydroxybutyrate is particularly high. With hepatic dysfunction, there may be hyperbilirubinemia.

Pathologic examination has generally revealed microvesicular and macrovesicular accumulation of fat in liver, skeletal muscle, and heart, but necrotic myopathy without accumulation of lipid has also been described [25] as has a predominance of type 1, slow oxidative muscle fibers. Hepatic cirrhosis has also been observed. Electron microscopy has revealed condensation of mitochondrial matrix and widening of crystal spaces [19, 29].

The diagnosis is most often suggested by the findings of large amounts of 3-hydroxydicarboxylic acid in the urine, or by the determination of the acylcarnitine profile in the blood. On gas chromatography-mass spectrometry (GCMS) organic acid analysis of the urine, the key compounds are hydroxy acids of up to 14 carbons [30], but medium chain dicarboxylic and 3-hydroxydicarboxylic acids are also found [2, 29]. Quantification in organic acid analysis is important in this condition as in others, for 3-hydroxydecanedioic acid and other dicarboxylic acids may be found in the urine in elevated amounts in ketosis, but in smaller quantity than in LCHAD deficiency [31]. Any of these abnormalities may become normal during an interim period of health between acute episodes. We have followed a patient in whom 3-hydroxyadipic acid is the only organic acid marker of the disease, even at times of acute rhabdomyolysis. This compound may be elevated by ketosis, but of course patients with this disease do not become ketotic. Assessment of the acylcarnitine profile of the plasma should reveal 3-hydroxyacid derivatives of the C16, C18, and C18:1 species (Figure 40.4) [32]. In addition, the 3-hydroxyacylcarnitines of C14, C14:1, and C18:1 are found [33], as well as the long chain acylcarnitines of C12, C14:1, C14, C16, C18:2, and C18:1. Over 85 percent of patients could be identified by elevation of hydrox-C18:1 over the 95th percentile of controls. High levels of endogenous long chain acylcarnitines in erythrocytes make blood spots much less reliable than plasma. The acylcarnitine profiles of total TFP deficiency and isolated LCHAD and LKAT deficiencies are indistinguishable. Oral loading with 3-phenylpropionate leads to the excretion of 3-hydroxyphenylpropionate, indicating the site of the defect [15].

GENETICS AND PATHOGENESIS

LCHAD deficiency is transmitted as an autosomal recessive trait. The molecular defect is in the mitochondrial

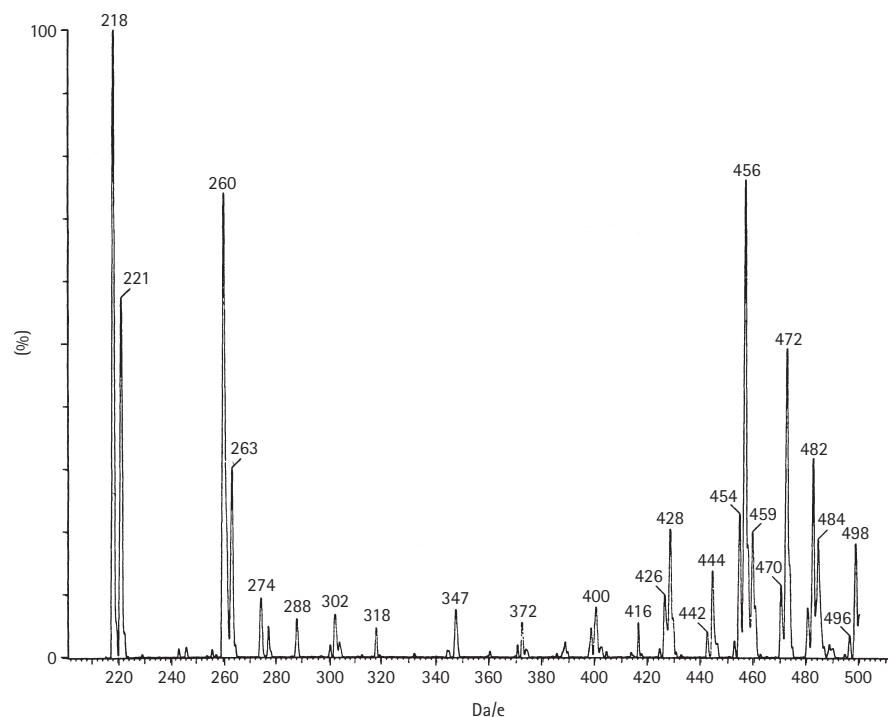


Figure 40.4 Acylcarnitine profile of the blood plasma of a patient with long chain L-3-hydroxyacyl CoA dehydrogenase (LCHAD) deficiency. Key compounds were the 3-hydroxyl derivatives of C14, C16, and C18. (Illustration provided by Jon Gangoiti of UCSD.)

trifunctional protein, which contains activities of LCHAD, 2-enoylCoA hydratase, and 3-oxoacylCoA thiolase. It differs from the trifunctional enzyme found in peroxisomes in structure and function [3], and is capable by itself of catalyzing the three sequential steps of β -oxidation. In some patients, there is defective activity of all three activities of the protein [27], but in most of the patients deficiency is isolated to LCHAD. Isolated deficiency of the thiolase is even less common [9].

The diagnosis may be confirmed by study of the oxidation of ^{14}C -labeled myristic (C14:0) and palmitic (C16:0) acids by lymphocytes [34] or fibroblasts, or by mutational analysis [12]. Acylcarnitine profiling with ^{13}C -labeled or ^{14}C -labeled substrate is not different in total TFP deficiency and isolated LCHAD or LKAT deficiencies [9]. The enzyme has usually been measured in fibroblasts in the reverse direction, with 3-oxopalmitoylCoA as substrate and measurement of the decrease in absorbance at 340 nm of the NADH electron donor. In some patients, activity is undetectable [18, 35], but since the SCHAD enzyme has some activity against longer fatty acids, activity is usually about 15–35 percent of control [2, 13, 18, 36]. Assay in the presence of antibody against the SCHAD protein generally gives LCHAD activity values less than 10 percent of control [2, 13, 18]. Enzyme activity of LKAT was measured with 3-ketopalmitoylCoA as substrate [9]. In rare instances, cross-reacting material (CRM) for TFP is virtually undetectable, and activity of the three enzymes is deficient, but in most instances immunoreactive MTP is normal and activity of only LCHAD is deficient [37]. Indication of the diagnosis has also been made by incubation of fibroblasts with palmitate and analysis of the medium for free

3-hydroxyacids [38]. Levels of 3-OHC14 and 3-OHC16 were increased 11- and 14-fold.

Intermediate activity in fibroblasts has been consistent with heterozygosity. Prenatal diagnosis can be made by enzyme analysis and by mutational analysis [39].

The gene for the α protein has been cloned and localized to chromosome 2p23.3-24.1, and the common mutation has been identified [6]. This G1528C mutation changes the glutamate 510 to glutamine. A simple polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) method for the detection of this mutation simplifies diagnosis and carrier detection [37]. Approximately 87 percent of chromosomes of patients with LCHAD deficiency have been found to carry the G1528C mutation [6–8, 37]. This mutation has frequently been found in infants of mothers with acute fatty liver of pregnancy [39, 40]. Expression studies indicated that the mutation induces loss of LCHAD activity [6]. Other mutations have been detected, usually in compound with G1528C. These include C1132T, which changes glutamine 342 to stop [40]. The mutation has also been designated at amino acid 474 (E474Q) [40]. Homozygosity for G1528C has been reported to lead to severe disease and death in early infancy [16, 34]. An infant with neonatal hypoglycemia and death in infancy had two different splice site mutations following exon 3 [41]. An infant whose mother had acute fatty liver of pregnancy was a compound of C1678T, which converted arginine 524 to stop and TFP deficiency with the common LCHAD mutation [42]. The gene for the β subunit has been localized also to chromosome 2p23 [43]. Patients with other than the G1528C mutations are among those with complete MTP deficiency and cardiomyopathy or

neuropathy. In the patient with isolated LKAT deficiency, two mutations were found in the *HADHB* gene, c.185G>A (p.R62H) in exon 4 and c.1292T>C (F431S) in exon 15 [9].

In two unrelated patients, mutations were found for the first time in the *HADHB* gene [44]. It appears that both normal α and β are important for stabilization of the trifunctional protein. Lethal disease was reported in a case of uniparental disomy of chromosome 2 leading to homozygous mutation in *HADHA* [45]. A knock-out mouse lacking the α and β subunits of MTP has neonatal hypoglycemia and sudden death [46].

The lactic acidemia observed so regularly in this disease may result from inhibition by accumulated long chain acyl CoA esters of the pyruvate dehydrogenase complex [47] or mitochondrial carriers, changing NADH/NAD ratios, or oxidative phosphorylation.

TREATMENT

The avoidance of fasting is important in the management of all patients with disorders of fatty acid oxidation, including LCHAD deficiency. The use of uncooked starch is an important adjunct to therapy. The addition of medium chain triglyceride (MCT) to the regimen has been reported to be therapeutic [1, 13, 14, 22]. Dosage has been 1.5 g/kg. Treatment with MCT was followed by improvement almost to normal in dicarboxylic aciduria, as well as a return to normal of the plasma level of long chain acyl carnitines [14]. Carnitine therapy restored to normal the level of free carnitine in plasma, but increased the concentrations of long chain acyl carnitines. Although many patients have improved, not all have. Peripheral neuropathy and retinopathy do not appear to benefit from MCT and dietary treatment.

Carnitine has been employed in doses approximating 50–100 mg/kg per day. Its use has become debatable because of concern that long chain acyl carnitine esters may be toxic, and some reports have suggested that carnitine-treated patients have done worse [13, 19, 48]. However, in the largest study, approximately half the patients were treated with carnitine, and no ill effect could be demonstrated [8]. In our view the use of carnitine in this disease makes sense. Riboflavin has been given in doses of 75–100 mg/day.

Dietary restriction of long chain fats in this disorder appears prudent, but it has been followed with highly variable stringency. The development of retinal degeneration has led to the hypothesis that this might be due to a shortage of essential fatty acids, such as linoleic and linolenic acids, sources of docosahexanoic acid (DHA), which is important in neural and retinal development. Monkeys deficient in DHA have had retinal degeneration [49], and there has been evidence of retinal dysfunction in premature infants that has been related to DHA [50]. DHA levels have been found to be low in patients with LCHAD deficiency [51]. For these reasons,

DHA supplementation has been initiated in patients with LCHAD deficiency [26] and this approach has increased levels of DHA in the blood. In four patients studied, there was electrophysiological evidence of visual improvement. An 11-year-old boy with LCHAD peripheral neuropathy had improved nerve conduction data after 12 months of treatment with cod liver oil, which is high in DHA [52]. Monitoring of change in the acylcarnitine profile has been reported to be useful in overall management, especially in MCT supplementation [33].

Treatment with creatine has been reported to be followed by a decrease in muscle pains and improved levels of CK [53].

REFERENCES

- Glasgow AM, Engel AG, Bier DM *et al.* Hypoglycemia hepatic dysfunction muscle weakness cardiomyopathy free carnitine deficiency and long-chain acylcarnitine excess responsive to medium chain triglyceride diet. *Pediatr Res* 1983; **17**: 319.
- Hale DE, Thorpe C, Braat K *et al.* The L-3-hydroxyacyl CoA dehydrogenase deficiency. In: Tanaka K, Coates PM (eds). *Fatty Acid Oxidation: Clinical Biochemical and Molecular Aspects*. New York: Alan R Liss, 1990: 503.
- Carpenter K, Pollitt RJ, Middleton B. Human liver long-chain 3-hydroxyacyl-coenzyme A dehydrogenase is a multifunctional membrane-bound beta-oxidation enzyme of mitochondria. *Biochem Biophys Res Commun* 1992; **183**: 433.
- Uchida Y, Izai K, Orii T, Hashimoto T. Novel fatty acid β -oxidation enzymes in rat liver mitochondria. II. Purification and properties of enoyl-coenzyme A (CoA) hydratase/3-hydroxyacyl CoA dehydrogenase/3-ketoacyl-CoA thiolase trifunctional protein. *J Biol Chem* 1992; **267**: 1034.
- Kamijo T, Aoyama T, Komiyama A, Hashimoto T. Structural analysis of cDNAs for subunits of human mitochondrial fatty acid beta-oxidation trifunctional protein. *Biochem Biophys Res Commun* 1994; **199**: 818.
- Ijlst L, Ruiter JPN, Hoovers JMN *et al.* Common missense mutation G1528C in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: characterization and expression of the mutant protein mutation analysis on genomic DNA and chromosomal localization of the mitochondrial trifunctional protein alpha subunit gene. *J Clin Invest* 1996; **98**: 1028.
- Ijlst L, Wanders RJA, Ushikubo S *et al.* Molecular basis of long-chain 3-hydroxyacyl-CoA deficiency: identification of the major disease-causing mutation in the alpha-subunit of the mitochondrial trifunctional protein. *Biochim Biophys Acta* 1994; **1215**: 347.
- den Boer MEJ, Wanders RJA, Morris AAM *et al.* Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: clinical presentation and follow-up of 50 patients. *Pediatrics* 2002; **109**: 99.
- Das AM, Illsinger S, Lucke T *et al.* Isolated mitochondrial long chain ketoacyl-CoA thiolase deficiency resulting from mutations in the HADHB gene. *Clin Chem* 2006; **52**: 530.

10. Poll-The BT, Bonnefont JP, Ogre H *et al.* Familial hypoketotic hypoglycemia associated with peripheral neuropathy pigmentary retinopathy and C₆-C₁₄ hydroxycarboxylic aciduria. A new defect in fatty acid oxidation? *J Inherit Metab Dis* 1988; **11**: 183.
11. Wanders RJA, Ijlst L, Duran M *et al.* Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency: different clinical expression in three unrelated patients. *J Inherit Metab Dis* 1991; **14**: 325.
12. Den Boer MEJ, Dionisi-Vici C, Chakrapani A *et al.* Mitochondrial trifunctional protein deficiency: a severe fatty acid oxidation disorder with cardiac and neurologic involvement. *J Pediatr* 2003; **142**: 684.
13. Jackson S, Bartlett K, Land J *et al.* Long-chain 3-hydroxy-acyl-CoA dehydrogenase deficiency. *Pediatric Res* 1991; **19**: 77.
14. Duran M, Wanders RJA, de Jaguar JP *et al.* 3-Hydroxydicarboxylic aciduria due to long chain 3-hydroxyacyl coenzyme A dehydrogenase deficiency associated with sudden neonatal death: protective effect of medium-chain triglyceride treatment. *Eur J Pediatr* 1991; **150**: 190.
15. Wanders JFA, Duran M, Ijlst L. Sudden infant death and long chain 3-hydroxyacyl CoA dehydrogenase. *Lancet* 1989; **2**: 52.
16. Hintz SR, Enns GM, Schelley S, Hoyme HE. Catastrophic presentation of long-chain 3-hydroxyacyl coenzyme A dehydrogenase (LCHAD) deficiency in early infancy. *Clin Res* 2001; **49**: 53A.
17. Mardsen D, Nyhan WL, Barshop BA. Creatine kinase and uric acid: early warning for metabolic imbalance resulting from disorders of fatty acid oxidation. *Eur J Pediatr* 2001; **160**: 599.
18. Dionisi-Vici C, Burlina AB, Bertini E *et al.* Progressive neuropathy and recurrent myoglobinuria in a child with long-chain 3-hydroxy-acyl-coenzyme A dehydrogenase deficiency. *J Pediatr* 1991; **118**: 744.
19. Rocchiccioli F, Wanders RJA, Aubourg P *et al.* Deficiency of long chain 3-hydroxyacyl CoA dehydrogenase: a cause of lethal myopathy and cardiomyopathy in early childhood. *Pediatr Res* 1990; **28**: 657.
20. Pohorecka M, Zuk M, Gradowska W *et al.* Cardiac abnormalities in 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency – report of 11 cases. *J Inherit Metab Dis* 2001; **24**: 70.
21. Ibdah JA, Dasouki MJ, Strauss AM *et al.* Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; variable expressivity of maternal illness during pregnancy and unusual presentation with infantile cholestasis and hypocalcaemia. *J Inherit Metab Dis* 1999; **2**: 811.
22. Losty HC, Shortland G, Olpin S, Pollitt RJ. Long chain hydroxyacyl CoA dehydrogenase deficiency – two further cases with obstetric complications. *Proc SSIEM* 1995; **34**: 106.
23. Tyni T, Kivela T, Lappi M *et al.* Ophthalmologic findings in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency caused by the G1528C mutation: a new type of hereditary metabolic chorioretinopathy. *Ophthalmology* 1998; **105**: 810.
24. Jackson S, Kler RS, Bartlett K *et al.* Combined defect of long-chain 3-hydroxyacyl-CoA dehydrogenase 2-enoyl-CoA hydratase and 3-oxoacyl CoA thiolase. In: Tanaka K, Coates P (eds). *New Developments in Fatty Acid Oxidation*. New York: Wiley-Liss, 1992: 327.
25. Bertini E, Dionisi-Vici C, Garavaglia B *et al.* Peripheral sensory-motor polyneuropathy pigmentary retinopathy and fatal cardiomyopathy in long-chain 3-hydroxy-acyl-CoA dehydrogenase deficiency. *Eur J Pediatr* 1992; **151**: 121.
26. Harding CO, Gillingham MB, van Calcar SC *et al.* Docosahexaenoic acid and retinal function in children with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 1999; **22**: 276.
27. Schaefer J, Jackson S, Dick D, Turnbull DM. Trifunctional enzyme deficiency: adult presentation of a usually fatal β -oxidation defect. *Proc SSIEM* 1996; **34**: 103.
28. Purevsuren J, Fukao T, Hasegawa Y *et al.* Clinical and molecular aspects of Japanese patients with mitochondrial trifunctional protein deficiency. *Mol Genet Metab* 2009; **98**: 372.
29. Kelley RJ, Morton DH. 3-Hydroxyoctanoic aciduria: identification of a new organic acid in the urine of a patient with non-ketotic hypoglycemia. *Clin Chim Acta* 1988; **175**: 19.
30. Politt RJ, Losty H, Westwood A. 3-Hydroxydicarboxylic aciduria: a distinctive type of intermittent dicarboxylic aciduria of possible diagnostic significance. *J Inherit Metab Dis* 1987; **10**: 226.
31. Greter J, Lindstedt S, Seeman H, Steen G. 3-Hydroxydecanedioic acid and related homologues. Urinary metabolites in ketoacidosis. *Clin Chem* 1980; **26**: 261.
32. Millington DS, Terada N, Chace DH *et al.* The role of tandem mass spectrometry in the diagnosis of fatty acid oxidation disorders. In: Coates PM, Tanaka K (eds). *New Developments in Fatty Acid Oxidation*. New York: Wiley-Liss, 1992: 339.
33. Van Hove JLK, Kahler SG, Feezor MD *et al.* Acylcarnitines in plasma and blood spots of patients with long-chain 3-hydroxyacylcoenzyme A dehydrogenase deficiency. *J Inherit Metab Dis* 2000; **23**: 571.
34. den Boer MEJ, Akkurt EK, Wijburg FA *et al.* Rapid diagnostic approach in LCHAD deficiency. *J Inherit Metab Dis* 1996; **19**(Suppl. 1): 110.
35. Tserng K-Y, Jin S-J, Kerr DS, Hoppel CL. Urinary 3-hydroxydicarboxylic acids in pathophysiology of metabolic disorders with dicarboxylic aciduria. *Metabolism* 1991; **40**: 676.
36. Przyrembel H, Jakobs C, Ijlst L *et al.* Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 1991; **14**: 674.
37. Ijlst L, Ruiter JPN, Oostveen W, Wanders RJA. Long-chain 3-hydroxy-acyl-CoA dehydrogenase deficiency: new mutations and the development of a simple PCR-RFLP method to detect the G1528C mutation in blood spots. *J Inherit Metab Dis* 1996; **19**(Suppl. 1): 109.
38. Jones PM, Moffitt M, Joseph D *et al.* Accumulation of free 3-hydroxy fatty acids in the culture media of fibroblasts from patients deficient in long-chain L-3-hydroxyacyl-CoA dehydrogenase: a useful diagnostic aid. *Clin Chem* 2001; **47**: 1190.
39. Ibdah JA, Bennett MJ, Zhao Y *et al.* Effects of fetal genotype on pregnancy outcome and validity of molecular prenatal diagnosis in families with mutations in mitochondrial trifunctional protein. *Am J Hum Genet* 1999; **65**: A45.

40. Sims HF, Brackett JC, Powell CK *et al.* The molecular basis of pediatric long chain 3-hydroxyacyl-CoA dehydrogenase deficiency associated with maternal acute fatty liver of pregnancy. *Proc Natl Acad Sci USA* 1995; **92**: 841.
41. Brackett JC, Sims HF, Rinaldo P *et al.* Two alpha subunit donor splice site mutations cause human trifunctional protein deficiency. *J Clin Invest* 1995; **95**: 2076.
42. Isaacs JD, Sims HF, Powell CK *et al.* Maternal acute fatty liver of pregnancy associated with fetal trifunctional protein deficiency: molecular characterization of a novel maternal mutant allele. *Pediatr Res* 1996; **40**: 393.
43. Yang BZ, Heng HH, Ding JH, Roe CR. The genes for the a and b subunits of the mitochondrial trifunctional protein are both located in the same region of human chromosome 2p23. *Genomics* 1996; **37**: 141.
44. Ushikubo S, Aoyama T, Kamijo T *et al.* Molecular characterization of mitochondrial trifunctional protein deficiency: formation of the enzyme complex is important for stabilization of both alpha-and beta-subunits. *Am J Hum Genet* 1996; **58**: 979.
45. Spiekerkoetter U, Eeds A, Yue Z *et al.* Uniparental disomy of chromosome 2 resulting in lethal trifunctional protein deficiency due to homozygous alpha-subunit mutations. *Hum Mutat* 2002; **20**: 447.
46. Ibdah JA, Paul H, Zhao Y *et al.* Lack of mitochondrial trifunctional protein in mice causes neonatal hypoglycemia and sudden death. *J Clin Invest* 2001; **107**: 1403.
47. Moore KH, Dandurand DM, Kiechle FL. Fasting-induced alterations in mitochondrial palmitoyl-CoA metabolism may inhibit adipocyte pyruvate dehydrogenase activity. *Int J Biochem* 1992; **24**: 809.
48. Tyni T, Palotie A, Viinikka L *et al.* Long-chain 3-hydroxyacyl coenzyme A dehydrogenase deficiency with the G1528C mutation: clinical presentation of 13 patients. *J Pediatr* 1997; **130**: 67.
49. Neuringer M, Connor WE, Lin DS *et al.* Biochemical and functional effects of prenatal and postnatal omega-3 fatty acid deficiency on retina and brain in rhesus monkeys. *Proc Natl Acad Sci USA* 1986; **83**: 4021.
50. Uauy R, Hoffman DR, Birch EE *et al.* Safety and efficacy of omega-3-fatty acids in nutrition of premature infants – soy oil and marine oil supplementation. *J Pediatr* 1994; **124**: 612.
51. Gillingham M, Van Calcar S, Ney D *et al.* Dietary management of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD). A case report and survey. *J Inherit Metab Dis* 1999; **22**: 23.
52. Tein I, Vajsar J, MacMillan L, Sherwood WG. Long-chain L-3-hydroxyacyl-coenzyme A dehydrogenase deficiency neuropathy: response to cod liver oil. *Neurology* 1999; **52**: 640.
53. Shortland GJ, Schmidt M, Losty H, Leonard JV. LCHAD deficiency treated with creatine. *J Inherit Metab Dis* 2001; **24**(Suppl. 1): 71.

Short-chain acyl CoA dehydrogenase deficiency

ZARAZUELA ZOLKIPLI

Introduction	302	Treatment	306
Clinical abnormalities	304	References	306
Genetics and pathogenesis	305		

MAJOR PHENOTYPIC EXPRESSION

Predominantly neurological phenotype of hypotonia, myopathy, developmental delay, microcephaly, seizures and failure to thrive.

INTRODUCTION

Short-chain acyl CoA dehydrogenase (SCAD) deficiency is an autosomal recessive short-chain fatty acid oxidation disorder [1]. An estimated birth prevalence of 1:33,000–1:50,000 was reported, based on high C4-carnitine levels on newborn screening, and the presence of *ACADS* gene mutations on both alleles [2, 3]. Short-chain acyl-CoA dehydrogenase is a member of the acyl-CoA dehydrogenase (ACAD) family of mitochondrial enzymes of the fatty acid β -oxidation pathway [4]. SCAD catalyzes the dehydrogenation of the fatty acylCoA compounds of chain length 4 to 6 carbons (Figure 41.1) [5, 6], transferring electrons to electron transfer flavoprotein

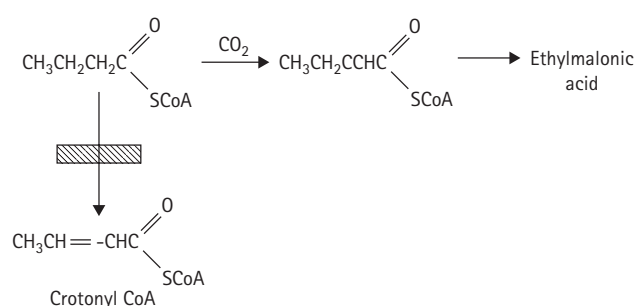


Figure 41.1 The short-chain acyl-CoA dehydrogenase reaction with butyryl CoA as a substrate. The conversion to ethylmalonylCoA is catalyzed by propionyl CoA carboxylase.

(ETF). SCAD is a tetrameric mitochondrial flavoenzyme, consisting of four subunits which are nuclear encoded and synthesized in the cytosol as precursor proteins. These are subsequently imported into the mitochondrial matrix to be proteolytically processed, folded, and assembled into the biologically active SCAD homotetramer (168 Da) [7]. The SCAD enzyme functions in the mitochondria [4, 7]. Each SCAD monomer contains one molecule of its cofactor, flavin adenine dinucleotide (FAD) [8]. FAD binding to the SCAD enzyme is important for its catalytic activity, folding, assembly, and/or stability [9–11]. The optimum substrate of the SCAD enzyme is butyrylCoA (C4-CoA); none of the other ACADs is active on this substrate *in vivo*. Deficiency of SCAD results in accumulation of butyrylCoA, which can be converted to butyrylcarnitine [12], butyrylglycine, and carboxylated by propionylCoA carboxylase to ethylmalonic acid [13]. The biochemical hallmark of SCAD deficiency is therefore raised levels of butyrylcarnitine which is evident on plasma acylcarnitine analysis, and ethylmalonic acid (EMA) which is observed on measurement of urine organic acids. It is noteworthy that elevated EMA is not specific to primary SCAD deficiency, as it is also elevated in ethylmalonic encephalopathy (Chapter 107) [14, 15], respiratory chain defects (Chapters 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, and 56) [16–18] and multiple acyl-CoA dehydrogenase deficiency (Chapter 44) [19]. EMA is therefore considered a nonspecific biochemical marker of SCAD enzyme dysfunction [20]. It does not correlate well with the severity of SCAD enzyme deficiency [1].

After the first patients with SCAD deficiency were reported [12, 21, 22], human SCAD cDNA was cloned by Naito *et al.* [8] based on its homology to the rat sequence. Subsequently, two pathogenic mutations were confirmed by Naito *et al.* at positions 136 and 319 of the coding region of the SCAD gene, which resulted in substitution of Arginine 22 and Arginine 83 with tryptophan (Trp) and cysteine (Cys), respectively [23]. Subsequently, the SCAD-encoding gene, *ACADS*, was localized to the terminal region of the long arm of chromosome 12, spanning approximately 13 kb, and consisting of 10 exons [24]. In the literature, approximately 38 different *ACADS* mutations have been described in patients with SCAD deficiency. Among those reported are c.136C>T, c.319C>T [23], c.1147C>T, c.274G>T, c.529C>T [25], c. 268G>A, c.575C>T, c.973C>T, c.1058C>T, c.1138C>T [20], c.332C>T, c.409C>T [26], c.417G>C, c.1095G>T [27], c.1138C>T, c.1058C>T, c.989G>A, c.988C>T, c.1170C>G, c.136C>T [3], and c.256G>T, c.820G>A, c.826G>A, c.1108A>G [1]. These rare inactivating mutations, most of which are missense, lead to a complete deficiency of SCAD activity.

There are two common *ACADS* variants which have been identified in patients with SCAD deficiency, c.625G>A (p.Glycine (Gly) 209 (Serine) S) and c.511C>T (p.Arg171Trp). These common variations are over-represented in homozygous or compound heterozygous form in up to 69 percent of patients with elevated levels of ethylmalonic acid (>18 mmol/mol creatinine), but are also found in 14 percent of the general population [20, 25, 28]. In the United States, analysis of 694 newborn blood spots revealed an allele frequency of 22 percent for the c.625G>A variant and 3 percent for the c.511C>T [29]. These variants were detected in either homozygous or compound heterozygous form in 7 percent of the study population. In another study on newborn screening blood spots in the Netherlands, analysis of 1036 screening cards revealed 5.5 percent homozygosity and 31.3 percent heterozygosity for the c.625G>A variant [30].

The majority of patients with SCAD deficiency are homozygotes or compound heterozygotes for two of the common *ACADS* gene variants (c.625A/625A, c.511T/511T, c.625A/511T) or have a combination of these variants on one allele, with an inactivating mutation on the other allele [1, 3, 31, 32]. Corydon *et al.* [20] reported a study of ten patients with ethylmalonic aciduria and deficiency of SCAD activity in fibroblasts. Sequence analysis revealed only one patient with a pathogenic mutation on both alleles. Five patients were doubly heterozygous for a pathogenic mutation on one allele, and common variation 625G>A in the other, while four other patients had either c.625G>A or c.511C>T on each allele. Waisbren *et al.* [32] described 14 patients with SCAD deficiency, of whom eight were identified from newborn screening, and six were patients identified on clinical presentation. All of the clinically identified children were homozygotes or compound heterozygotes

for the common variants, except for one patient who also carried a 136C>T mutation. Pedersen *et al.* [1] reported the *ACADS* gene variation spectrum in 114 patients with SCAD deficiency identified on the basis of ethylmalonic aciduria, elevated butyrylcarnitine in plasma, and/or fibroblasts, and decreased SCAD enzyme activity in fibroblasts or muscle. All but four patients were clinically symptomatic. The c.625G>A and the c.511C>T variations were present in 67 and 8 percent of the investigated alleles, respectively, compared to 21 and 8 percent in 100 alleles from Danish controls. Eleven of the 114 patients carried rare *ACADS* gene variations on both alleles, 39 were compound heterozygotes for a rare and a common variation, and 64 were homozygous or compound heterozygous for two common variations. Tein *et al.* [31] reported ten patients of Ashkenazi Jewish ancestry with variable neuromuscular symptoms, three out of the ten patients were homozygous for c.319C>T, the remaining seven had C.319C>T on one allele, and common variant c.625G>A on the other. The authors performed a population screening survey of c.319C>T in 105 individuals of Ashkenazi Jewish descent and found a carrier frequency of 1:15 and a 1:900 homozygote frequency, suggesting a likely founder effect. The incidence of SCAD deficiency, as well as the other diseases of fatty acid oxidation is reportedly lower in Asian populations, in comparison to Caucasians [33]. The frequency of the c.625G>A variant in the Hispanic population (30 percent) is reported to be significantly higher than that of the African-American (9 percent) and Asian (13 percent) subpopulations [30].

The role of these common variants in the pathogenesis of SCAD deficiency remains poorly understood. The frequency of homozygosity for one of these variations in the general population suggests that they are not sufficient alone to cause SCAD disease. Alternatively, they are likely to confer susceptibility [20, 25, 34]. It has been suggested that these variants lead to disease in combination with genetic and/or environmental factors [20]. Under specific conditions of stress such as elevated temperature, or in conjunction with an inactivating mutation or any other gene modifier, these variants could lead to reduced SCAD activity and result in SCAD disease [25].

The presence of SCAD variants and/or biochemical evidence of SCAD enzyme dysfunction in apparently unaffected individuals, as has been shown in family members of probands [1, 28], or asymptomatic patients identified on newborn screening, have raised questions of their clinical relevance [3, 35]. The benefits of an early diagnosis is unclear [36, 37]. The natural history remains poorly understood. There is insufficient evidence for optimal treatment [38]. The American College of Medical Genetics published an expert panel report which recommended the exclusion of SCAD deficiency from the core panel of fatty acid oxidation disorders screened. However, since SCAD deficiency is in a differential of the core diseases, it was advised that SCAD deficiency be

retained as a secondary target [33]. Newborn screening for SCAD deficiency is performed in 35 of 51 states, as well as in Austria and Belgium [38]. In the United Kingdom, Denmark, and the Netherlands, SCAD deficiency is not included in the programs of newborn screening [38].

CLINICAL ABNORMALITIES

The reported age of onset is from the neonatal period [21] to adulthood [20]. In most cases, onset is before five years of age [1, 3, 28]. In the largest series to date of patients with SCAD deficiency, Pedersen *et al.* [1] reported the age range of onset to be from 0 to 50 years of age, of whom 25 percent presented on the first day of life, 61 percent in the first year of life, and 4 percent after the age of ten years. The initial reported patients with enzymatically confirmed SCAD deficiency were of neonatal onset, one of which was fatal in the first week [21]. Since then, the clinical spectrum of SCAD deficiency has been extended to milder presentations [39]. The clinical course is unpredictable. Patients with SCAD deficiency have been reported who have had transient symptoms [3, 32], as well as patients who have improved to baseline over time [1, 3]. The spectrum of clinical features is difficult to correlate to the level of SCAD enzyme activity [3, 34], and there are no consistent genotype–clinical phenotype correlations [1, 3, 25]. There are also patients with SCAD deficiency who remain symptom free for many years after diagnosis,

as reported in newborn screening follow-up studies [32, 40–42], or in family studies [28].

In contrast to the other ACAD deficiencies which are more likely to present with hypoketotic hypoglycemia, hepatic or cardiac dysfunction, the clinical features of SCAD are predominantly neurological [1, 25, 28, 43]. Developmental delay is the most common manifestation. Pedersen *et al.* [1] reported this in 69 percent of the 114 patients with SCAD deficiency. This was also described in other patient series [3, 28]. The other common symptoms are speech delay and hypotonia (Figure 41.2) [1, 28]. Other frequently reported features are seizures [1, 3], myopathy [1, 28], failure to thrive and feeding difficulties [1, 28], lethargy [28], and behavioral problems [3]. Some do have hypoglycemia [1, 3]. Less frequently seen are dysmorphic features, cardiomyopathy, intrauterine growth impairment, or respiratory distress [1]. There has been a single reported case of abnormal cortical gyration and hypoplastic corpus callosum [44]. Acute metabolic episodes were not reported commonly [28]. Waisbren *et al.* [32] reported five of 14 patients with SCAD deficiency diagnosed on newborn screening, whose mothers had acute fatty liver of pregnancy with pre-eclampsia and HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome. There had been one prior reported case [45].

An initial biochemical evaluation of SCAD deficiency should include urinary organic acid profile, plasma acylcarnitine profile, and plasma carnitine [46]. Organic acid analysis characteristically reveals increased excretion of ethylmalonic acid. It is pertinent to note that although an elevated urinary EMA is characteristic, it is not diagnostic of SCAD deficiency. At times of relative stress, methylsuccinic acid may be excreted in the urine [21]. Butyrylglycine and butyrylcarnitine may also be found in urine [47–49]. The urine can be normal at times of relative health. This underlines the intermittent nature of the excretion of EMA, which appears to be dependent upon the degree of intercurrent metabolic stress [1]. Butyrylcarnitine should be distinguished from isobutyrylcarnitine, which may be found in normal individuals [50], ethylmalonic encephalopathy [14], and in patients with a defect in branched-chain acyl CoA oxidation, isobutyryl CoA dehydrogenase deficiency [51]. These distinctions may be made by organic acid analysis, enzyme assay, mutational analysis, or study of the accumulation of labeled acylcarnitines *in vitro*.

In newborn screening programs, SCAD deficiency is detected by acylcarnitine measurement using tandem mass spectrometry (MS/MS) on plasma or blood spots. The key compound is C₄, butyrylcarnitine (Figure 41.3), but C₅ may also be elevated. The diagnosis may subsequently be confirmed by performing enzymatic assays in skin fibroblasts. ¹⁴C-butyrate uptake studies have previously served as a screening test for this disorder [52]. Determination of SCAD enzyme activity requires butyryl CoA as a substrate [53], and incubation with anti-medium-chain acyl CoA dehydrogenase (MCAD) antibody, in

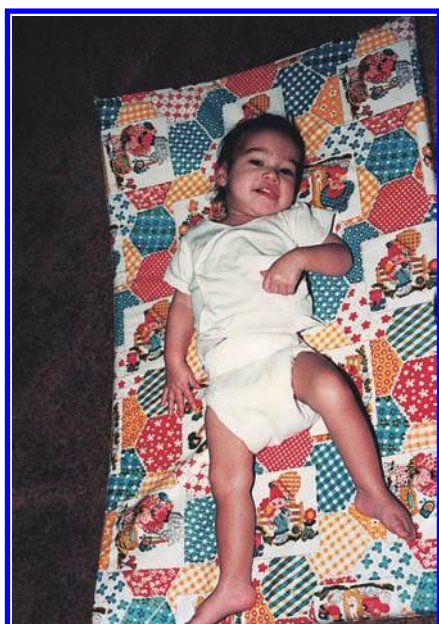


Figure 41.2 JG: An infant with short-chain acyl-CoA dehydrogenase deficiency. She was markedly hypotonic and had skeletal muscle weakness. Concentrations of free-carnitine in blood and muscle were low [1]. (Illustration was kindly provided by Dr Susan Winter, Fresno, California.)

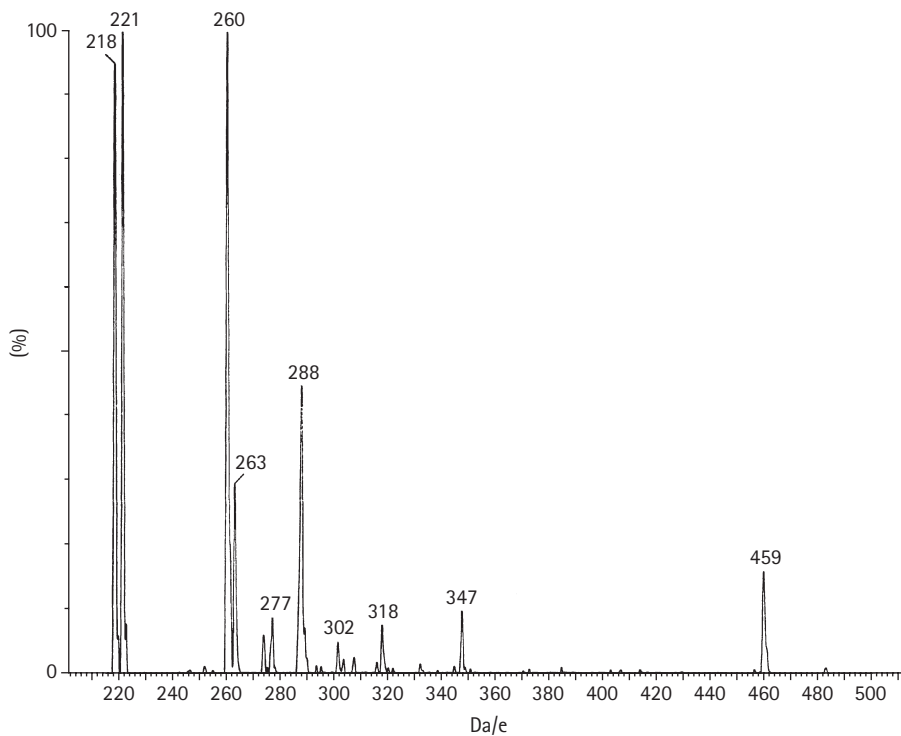


Figure 41.3 Acycarnitine tandem mass spectrometry profile of a three-year-old patient with short-chain acyl-CoA dehydrogenase deficiency. He presented with myopathy and hepatomegaly. He had episodes of hypoglycemia and lactic acidosis. He died of cardiomyopathy. The key compounds are 288 C4-carnitine and 302 C5-carnitine.

view of the overlapping activity of MCAD toward C4-C6 acylCoAs [21, 52, 54]. However, enzyme analysis in fibroblasts or lymphocytes has demonstrated high residual SCAD activity in some patients with SCAD deficiency [27, 55], or has given inconsistent results [20, 25]. Measurement in skeletal muscle is reported to be more reliable than skin fibroblasts [55]. Confirmation of the diagnosis of SCAD deficiency is typically by molecular analysis of the *ACADS* gene, with full sequencing if required.

GENETICS AND PATHOGENESIS

SCAD deficiency continues to be a major diagnostic management dilemma because of the marked genetic heterogeneity and clinical unpredictability of the *ACADS* gene spectrum, and the lack of correlation between genotypes, clinical phenotypes, and pathophysiology. Symptoms of SCAD deficiency are primarily neurological, and the reasons for the neurotoxicity are still not understood. Gregersen *et al.* [56] highlighted the expanse of the *ACADS* gene spectrum, compared to those of the other *ACADS*, such as MCAD and VLCAD. In addition to the extensive spectrum of inactivating variations, the two common variants c625G>A and c.511 C>T are found in up to 14 percent of the general population in homozygous or compound heterozygous form [25, 30]. In 293 symptomatic patients and 45 screened newborns, 58 *ACADS* variations were found, 50 of which were missense mutations and one was an in-frame deletion. In the symptomatic patient

group, 10 percent carried two rare inactivating mutations, 34 percent carried one inactivating variant on one allele, and a common variant on the other allele, and 56 percent were either homozygous or compound heterozygotes for a common variant. On the other hand, the proportion of patients in the screened newborn group who carried two inactivating variations was much higher at 65 percent. Thirteen percent carried one inactivating and one common variant, and 22 percent were homozygous or compound heterozygous for a common variant. It is not understood why a significant number of the screened newborns carrying rare inactivating mutations, or their family members carrying the same rare variants, would remain asymptomatic. Conversely, it is not clear why a higher proportion (56 percent) of symptomatic patients are either homozygous or compound heterozygotes for the common variations, most often the c.625G>A variant [1, 20, 25, 28].

Patients homozygous for inactivating mutations show more severe biochemical abnormalities with very low levels of SCAD activity [1, 28, 57, 58], compared to those with one inactivating variant and one common variant [55]. Patients who are homozygous or compound heterozygous for the common variants have either normal or variably decreased levels of SCAD activity [25]. This may explain why only 22 percent of screened newborns have the common variants, as some individuals who are homozygous or compound heterozygous for a common variant may not have butyrylcarnitine levels above the newborn screening cut-off level and remain unidentified [32]. Measurement of common variant proteins p.Gly209Ser, encoded by

c.625G>A and p.Arg147Trp, encoded by c.511C>T in *E. coli* at 37°C showed 86 and 69 percent activity of the mean wild-type SCAD value [20]. Expression of the p.Gly209Ser variant protein in Cos-7 cells revealed corresponding lower SCAD activity levels at higher temperature, reducing from 45 to 13 percent, with temperature increase from 37 to 41°C, respectively. The p.Arg147Trp SCAD protein in Cos-7 cells also showed decreasing activity, but from higher than normal levels at 37°C, reducing to 58 percent at 41°C. Catalytic function of the purified p.Gly209Ser variant protein in *E. coli* has been shown to be impaired compared to wild-type SCAD, whereas the p.Arg147Trp was similar to wild type [59]. Subsequently, *in vitro* import studies of inactivating variant proteins, including the common variant SCAD proteins, in isolated mitochondria from SCAD-deficient mice demonstrated an increased tendency to protein misfolding and aggregation [1, 57]. The mechanism whereby misfolding and aggregation leads to cellular toxicity has been thought to relate to the accumulation of toxic aggregates, or toxicity of soluble oligomeric species [60]. SCAD variant protein misfolding was also shown to be temperature dependent [1, 57]. This further underlines the relevance of environmental and/or genetic factors in the pathophysiology of SCAD disease. This is particularly relevant in the understanding of the pathophysiology of the susceptibility variants, c.625G>A and c.511C>T. Pedersen *et al.* [61] investigated fibroblast cell lines from ten symptomatic patients with c.625G>A homozygosity, compared to four asymptomatic individuals with the same c.625G>A homozygosity and control fibroblasts (n = 24) which were confirmed to have normal SCAD function without c.625G>A homozygosity. The symptomatic patient lines were shown to have significantly reduced levels of SCAD activity and reduced expression of SCAD mRNA. Superoxide dismutase 2 (SOD2), a major intramitochondrial antioxidant enzyme, was reduced on quantitative proteomic assay by nano-LC-MS/MS and confirmed by Western blot. There was also increased sensitivity to menadione-induced oxidative stress, compared to controls and healthy c.625G>A homozygote cells. This is consistent with increased sensitivity to oxidative stress in the c.625G>A patient cells. Conversely, the fibroblasts from healthy individuals with C.625G>A homozygosity revealed comparable levels of SCAD protein expression and activity to the control group, and instead had higher SOD2 expression on proteomic analysis and Western blot, in conjunction with increased resistance to menadione-induced oxidative stress. This suggested increased resistance to oxidative stress in cells from c.625G>A healthy individuals. Sequence analysis of all of the exons and a large part of the promoter in the SOD2 gene revealed identical haplotypes in all groups. It is speculated that SOD2 gene dysregulation could therefore be a factor. Antioxidant dysfunction and increased susceptibility to oxidative stress may therefore contribute to the pathophysiology of SCAD deficiency [61]. Furthermore, Schmidt *et al.* [62] reported that the

c.319C>T SCAD protein p.Arg83Cys was unable to attain the normal soluble conformation when expressed in an astrocytic cell line, and therefore prone to accumulate in the insoluble fraction. Concomitantly, fission of the mitochondrial network was evident, which was likely to be due to oxidative stress. This further supports the hypothesis of protein misfolding, oxidative stress, and mitochondrial dysfunction as contributory pathogenetic mechanisms.

TREATMENT

The efficacy of treatment is unclear. Patients have been treated with carnitine and restriction of dietary fat. Prolonged fasting should be avoided. Van Maldegem *et al.* [63] reported a lack of clinical improvement to high-dose riboflavin.

REFERENCES

1. Pedersen CB, Kølvrå S, Kølvrå A *et al.* The ACADS gene variation spectrum in 114 patients with short-chain acyl-CoA dehydrogenase (SCAD) deficiency is dominated by missense variations leading to protein misfolding at the cellular level. *Hum Genet* 2008; **124**: 43.
2. Zytkevich TH, Fitzgerald EF, Marsden D *et al.* Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a two-year summary from the New England Newborn Screening Program. *Clin Chem* 2001; **47**: 1945.
3. van Maldegem BT, Duran M, Wanders RJ *et al.* Clinical, biochemical, and genetic heterogeneity in short-chain acyl-coenzyme A dehydrogenase deficiency. *J Am Med Assoc* 2006; **296**: 943.
4. Matsubara Y, Indo Y, Naito E *et al.* Molecular cloning and nucleotide sequence of cDNAs encoding the precursors of rat long chain acyl-coenzyme A, short chain acyl-coenzyme A, and isovaleryl-coenzyme A dehydrogenases. Sequence homology of four enzymes of the acyl-CoA dehydrogenase family. *J Biol Chem* 1989; **264**: 16321.
5. Davidson B, Schulz H. Separation, properties, and regulation of acyl coenzyme A dehydrogenases from bovine heart and liver. *Arch Biochem Biophys* 1982; **213**: 155.
6. Shaw L, Engel PC. The purification and properties of ox liver short-chain acyl-CoA dehydrogenase. *Biochem J* 1984; **218**: 511.
7. Ikeda Y, Keese SM, Fenton WA *et al.* Biosynthesis of four rat liver mitochondrial acyl-CoA dehydrogenases: *in vitro* synthesis, import into mitochondria, and processing of their precursors in a cell-free system and in cultured cells. *Arch Biochem Biophys* 1987; **252**: 662.
8. Naito E, Ozasa H, Ikeda Y, Tanaka K. Molecular cloning and nucleotide sequence of complementary DNAs encoding human short chain acyl-coenzyme A dehydrogenase and the study of the molecular basis of human short chain acyl-coenzyme A dehydrogenase deficiency. *J Clin Invest* 1989; **83**: 1605.

9. Henriques BJ, Rodrigues JV, Olsen RK *et al.* Role of flavinylation in a mild variant of multiple acyl-CoA dehydrogenation deficiency: a molecular rationale for the effects of riboflavin supplementation. *J Biol Chem* 2009; **284**: 4222.
10. Saijo T, Tanaka K. Isoalloxazine ring of FAD is required for the formation of the core in the Hsp60-assisted folding of medium chain acyl-CoA dehydrogenase subunit into the assembly competent conformation in mitochondria. *J Biol Chem* 1995; **270**: 1899.
11. Nagao M, Tanaka K. FAD-dependent regulation of transcription, translation, post-translational processing, and post-processing stability of various mitochondrial acyl-CoA dehydrogenases and of electron transfer flavoprotein and the site of holoenzyme formation. *J Biol Chem* 1992; **267**: 17925.
12. Coates PM, Hale DE, Finocchiaro G *et al.* Genetic deficiency of short-chain acyl-coenzyme A dehydrogenase in cultured fibroblasts from a patient with muscle carnitine deficiency and severe skeletal muscle weakness. *J Clin Invest* 1988; **81**: 171.
13. Hegre CS, Halenz DR, Lane MD. The enzymatic carboxylation of butyryl coenzyme A. *J Am Chem Soc* 1959; **84**: 6526.
14. Burlina A, Dionisi-Vici C, Bennett MJ *et al.* A new syndrome with ethylmalonic aciduria and normal fatty acid oxidation in fibroblasts. *J Pediatr* 1994; **124**: 79.
15. Tiranti V, D'Adamo P, Briem E *et al.* Ethylmalonic encephalopathy is caused by mutations in ETHE1, a gene encoding a mitochondrial matrix protein. *Am J Hum Genet* 2004; **74**: 239.
16. Hoffmann GF, Hunneman DH, Jakobs C *et al.* Progressive fatal pancytopenia, psychomotor retardation and muscle carnitine deficiency in a child with ethylmalonic aciduria and ethylmalonic acidemia. *J Inherit Metab Dis* 1990; **13**: 337.
17. Lehnert W, Ruitenbeek W. Ethylmalonic aciduria associated with progressive neurological disease and partial cytochrome c oxidase deficiency. *J Inherit Metab Dis* 1993; **16**: 557.
18. Christensen E, Brandt NJ, Schmalbruch H *et al.* Muscle cytochrome c oxidase deficiency accompanied by a urinary organic acid pattern mimicking multiple acyl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 1993; **16**: 553.
19. Rhead WJ, Amendt BA. Electron-transferring flavoprotein deficiency in the multiple acyl-CoA dehydrogenation disorders, glutaric aciduria type II and ethylmalonic-adipic aciduria. *J Inherit Metab Dis* 1984; **7**: 99.
20. Corydon MJ, Vockley J, Rinaldo P *et al.* Role of common gene variations in the molecular pathogenesis of short-chain acyl-CoA dehydrogenase deficiency. *Pediatr Res* 2001; **49**: 18.
21. Amendt BA, Greene C, Sweetman L *et al.* Short-chain acyl-coenzyme A dehydrogenase deficiency. Clinical and biochemical studies in two patients. *J Clin Invest* 1987; **79**: 1303.
22. Bennett MJ, Gray RG, Isherwood DM *et al.* The diagnosis and biochemical investigation of a patient with a short chain fatty acid oxidation defect. *J Inherit Metab Dis* 1985; **8**: 135.
23. Naito E, Indo Y, Tanaka K. Identification of two variant short chain acyl-coenzyme A dehydrogenase alleles, each containing a different point mutation in a patient with short chain acyl-coenzyme A dehydrogenase deficiency. *J Clin Invest* 1990; **85**: 1575.
24. Corydon MJ, Andresen BS, Bross P *et al.* Structural organization of the human short-chain acyl-CoA dehydrogenase gene. *Mamm Genome* 1997; **8**: 922.
25. Gregersen N, Winter VS, Corydon MJ *et al.* Identification of four new mutations in the short-chain acyl-CoA dehydrogenase (SCAD) gene in two patients: one of the variant alleles, 511C→T, is present at an unexpectedly high frequency in the general population, as was the case for 625G→A, together conferring susceptibility to ethylmalonic aciduria. *Hum Mol Genet* 1998; **7**: 619.
26. Koeberl DD, Young SP, Gregersen NS *et al.* Rare disorders of metabolism with elevated butyryl- and isobutyryl-carnitine detected by tandem mass spectrometry newborn screening. *Pediatr Res* 2003; **54**: 219.
27. Seidel J, Streck S, Bellstedt K *et al.* Recurrent vomiting and ethylmalonic aciduria associated with rare mutations of the short-chain acyl-CoA dehydrogenase gene. *J Inherit Metab Dis* 2003; **26**: 37.
28. Corydon MJ, Gregersen N, Lehnert W *et al.* Ethylmalonic aciduria is associated with an amino acid variant of short chain acyl-coenzyme A dehydrogenase. *Pediatr Res* 1996; **39**: 1059.
29. Nagan N, Kruckeberg KE, Tauscher AL *et al.* The frequency of short-chain acyl-CoA dehydrogenase gene variants in the US population and correlation with the C(4)-acylcarnitine concentration in newborn blood spots. *Mol Genet Metab* 2003; **78**: 239.
30. van Maldegem BT, Waterham HR, Duran M *et al.* The 625G>A SCAD gene variant is common but not associated with increased C4-carnitine in newborn blood spots. *J Inherit Metab Dis* 2005; **28**: 557.
31. Tein I, Elpeleg O, Ben-Zeev B *et al.* Short-chain acyl-CoA dehydrogenase gene mutation (c.319C>T) presents with clinical heterogeneity and is candidate founder mutation in individuals of Ashkenazi Jewish origin. *Mol Genet Metab* 2008; **93**: 179.
32. Waisbren SE, Levy HL, Noble M *et al.* Short-chain acyl-CoA dehydrogenase (SCAD) deficiency: an examination of the medical and neurodevelopmental characteristics of 14 cases identified through newborn screening or clinical symptoms. *Mol Genet Metab* 2008; **95**: 39.
33. Lindner M, Hoffmann GF, Matern D. Newborn screening for disorders of fatty-acid oxidation: experience and recommendations from an expert meeting. *J Inherit Metab Dis* 2010; **33**: 521.
34. Gregersen N, Andresen BS, Bross P. Prevalent mutations in fatty acid oxidation disorders: diagnostic considerations. *Eur J Pediatr* 2000; **159**: S213.
35. Dietzen DJ, Rinaldo P, Whitley RJ *et al.* National academy of clinical biochemistry laboratory medicine practice guidelines: follow-up testing for metabolic disease identified by expanded newborn screening using tandem mass spectrometry; executive summary. *Clin Chem* 2009; **55**: 1615.
36. Wilcken B, Wiley V, Hammond J, Carpenter K. Screening newborns for inborn errors of metabolism by tandem mass spectrometry. *N Engl J Med* 2003; **348**: 2304.
37. Wilcken B, Wiley V. Newborn screening. *Pathology* 2008; **40**: 104.

38. American College of Medical Genetics Newborn Screening Expert Group. Newborn screening: toward a uniform screening panel and system – executive summary. *Pediatrics* 2006; **117**: S296.
39. Ribes A, Riudor E, Garavaglia B. Mild or absent clinical signs in twin sisters with short-chain acyl-CoA dehydrogenase deficiency. *Eur J Pediatr* 1998; **157**: 317.
40. Rhead WJ, Allain D, Van Calcar S *et al*. Short-chain acyl-coenzyme A dehydrogenase (SCAD) and 3-methylcrotonyl-CoA carboxylase (MCC) deficiencies: tandem mass spectrometry newborn screening detects many clinically benign cases. *J Inherit Metab Dis* 2002; **25**: 4 (Abstr.).
41. Jethva R, Ficicioglu C. Clinical outcomes of infants with short-chain acyl-coenzyme A dehydrogenase deficiency (SCADD) detected by newborn screening. *Mol Genet Metab* 2008; **95**: 241.
42. Wilcken B. The consequences of extended newborn screening programmes: do we know who needs treatment? *J Inherit Metab Dis* 2008; **22**: 173.
43. van Maldegem BT, Wanders RJ, Wijburg FA. Clinical aspects of short-chain acyl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 2010; **33**: 507.
44. Mikati MA, Chaaban HR, Karam PE, Krishnamoorthy KS. Brain malformation and infantile spasms in a SCAD deficiency patient. *Pediatr Neurol* 2007; **36**: 48.
45. Matern D, Hart P, Murtha AP *et al*. Acute fatty liver of pregnancy associated with short-chain acyl-coenzyme A dehydrogenase deficiency. *J Pediatr* 2001; **138**: 585.
46. Jethva R, Bennett MJ, Vockley J. Short-chain acyl-coenzyme A dehydrogenase deficiency. *Mol Genet Metab* 2008; **95**: 195.
47. Costa CG, Guérard WS, Struys EA *et al*. Quantitative analysis of urinary acylglycines for the diagnosis of beta-oxidation defects using GC-NCI-MS. *J Pharm Biomed Anal* 2000; **21**: 1215.
48. Bonafé L, Troxler H, Kuster T *et al*. Evaluation of urinary acylglycines by electrospray tandem mass spectrometry in mitochondrial energy metabolism defects and organic acidurias. *Mol Genet Metab* 2000; **69**: 302.
49. Sim KG, Hammond J, Wilcken B. Strategies for the diagnosis of mitochondrial fatty acid beta-oxidation disorders. *Clin Chim Acta* 2002; **323**: 37.
50. Schmidt-Sommerfeld E, Penn D, Kerner J, Bieber LL. Analysis of acylcarnitines in normal human urine with the radioisotopic exchange-high performance liquid chromatography (HPLC) method. *Clin Chim Acta* 1989; **181**: 231.
51. Roe CR, Cederbaum SD, Roe DS *et al*. Isolated isobutyryl-CoA dehydrogenase deficiency: an unrecognized defect in human valine metabolism. *Mol Genet Metab* 1998; **65**: 264.
52. Bhala A, Willi SM, Rinaldo P *et al*. Clinical and biochemical characterization of short-chain acyl-coenzyme A dehydrogenase deficiency. *J Pediatr* 1995; **126**: 910.
53. Vianey-Saban C, Divry P, Brivet M. Mitochondrial very-long-chain acyl-coenzyme A dehydrogenase deficiency: clinical characteristics and diagnostic considerations in 30 patients. *Clin Chim Acta* 1998; **269**: 43.
54. Wanders RJ, Vreken P, den Boer ME *et al*. Disorders of mitochondrial fatty acyl-CoA beta-oxidation. *J Inherit Metab Dis* 1999; **22**: 442.
55. Bok LA, Vreken P, Wijburg FA *et al*. Short-chain acyl-CoA dehydrogenase deficiency: studies in a large family adding to the complexity of the disorder. *Pediatrics* 2003; **112**: 1152.
56. Gregersen N, Andresen BS, Pedersen CB *et al*. Mitochondrial fatty acid oxidation defects--remaining challenges. *J Inherit Metab Dis* 2008; **31**: 643.
57. Pedersen CB, Bross P, Winter VS *et al*. Misfolding, degradation, and aggregation of variant proteins. The molecular pathogenesis of short chain acyl-CoA dehydrogenase (SCAD) deficiency. *J Biol Chem* 2003; **278**: 47449.
58. Shirao K, Okada S, Tajima G *et al*. Molecular pathogenesis of a novel mutation, G108D, in short-chain acyl-CoA dehydrogenase identified in subjects with short-chain acyl-CoA dehydrogenase deficiency. *Hum Genet* 2010; **127**: 619.
59. Nguyen TV, Riggs C, Babovic-Vuksanovic D *et al*. Purification and characterization of two polymorphic variants of short chain acyl-CoA dehydrogenase reveal reduction of catalytic activity and stability of the Gly185Ser enzyme. *Biochemistry* 2002; **41**: 11126.
60. Stefani M, Dobson CM. Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. *J Mol Med* 2003; **81**: 678.
61. Pedersen CB, Zolkipli Z, Vang S *et al*. Antioxidant dysfunction: potential risk for neurotoxicity in ethylmalonic aciduria. *J Inherit Metab Dis* 2010; **33**: 211.
62. Schmidt SP, Corydon TJ, Pedersen CB *et al*. Misfolding of short-chain acyl-CoA dehydrogenase leads to mitochondrial fission and oxidative stress. *Mol Genet Metab* 2010; **100**: 155.
63. van Maldegem BT, Duran M, Wanders RJ *et al*. Flavin adenine dinucleotide status and the effects of high-dose riboflavin treatment in short-chain acyl-CoA dehydrogenase deficiency. *Pediatr Res* 2010; **67**: 304.

3-HydroxyacylCoA dehydrogenase (short-chain 3-hydroxyacylCoA dehydrogenase) deficiency

Introduction	309	Treatment	310
Clinical abnormalities	309	References	310
Genetics and pathogenesis	310		

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia, recurrent myoglobinuria, encephalopathy and cardiomyopathy; or hyperketotic hypoglycemia, failure to thrive and hypotonia; elevated creatine kinase; dicarboxylic aciduria; and defective activity of HADH in muscle fibroblasts and leukocytes. Some patients with familial hyperinsulinemic hypoglycemia (HHF₄) have mutations in the *HADH* gene.

INTRODUCTION

Deficiency of hydroxyacylCoA dehydrogenase (HADH) (EC 1.1.1.35) was first described by Tein and colleagues [1] in a 16-year-old girl with recurrent myoglobinuria and hypoketotic hypoglycemia in whom HADH activity was markedly diminished in muscle, but normal in fibroblasts. In contrast, we and others have seen patients in whom enzyme activity was very low in cultured fibroblasts and freshly isolated leukocytes [2]. These patients have had what appeared to be ketotic hypoglycemia.

The enzyme, 3-hydroxyacyl CoA dehydrogenase, is a homodimer with 302 amino acids in each subunit [3–5] with activity against 3-hydroxyacylCoA esters of C4 to C16 length, but with greatest activity against C10 and less activity as the chain length increases (Figure 42.1). The cDNA for the gene has been cloned and sequenced [6, 7]

The diagram illustrates the enzymatic reaction catalyzed by 3-hydroxyacylCoA dehydrogenase. On the left, the substrate is 3-hydroxybutyryl-CoA, represented as $\text{CH}_3\text{CH}(\text{OH})\text{CH}_2\text{C}(=\text{O})\text{SCoA}$. This reacts with NAD^+ . A reversible arrow, indicated by a hatched box, points to the right. On the right, the products are 3-oxobutryl-CoA, represented as $\text{CH}_3\text{C}(=\text{O})\text{CH}_2\text{C}(=\text{O})\text{SCoA}$, and $\text{NADH} + \text{H}^+$.

Figure 42.1 The 3-hydroxyacylCoA dehydrogenase reaction. Substrates 3-hydroxybutyryl CoA and the ketoacid product. The enzyme catalyzes conversion of C₄ to C₁₆ esters.

and mapped to chromosome 4q22-26 [7]. It contains eight exons and spans 49 kb. The enzyme is synthesized with a leader peptide, which is removed after import into the mitochondria. Mutations have been identified [8].

CLINICAL ABNORMALITIES

The initial patient [1] with deficiency of HADH had episodic myoglobinuria and hypoketotic hypoglycemia, as expected for a disorder of fatty acid oxidation. There was also evidence of encephalopathy and hypertrophic dilated cardiomyopathy.

Our patient [2] had a neonatal presentation of difficulty with feeding, failure to thrive, and hypotonia. An elevated creatine phosphokinase (CPK) of 2000 U/L led to a muscle biopsy, which appeared normal. The CK was recorded as high as 5721 U/L. In response to fasting, she developed hypoglycemia of 38 mg/dL at 23 hours, but the concentration of 3-hydroxybutyrate in the blood was 2120 mmol/L, which was a brisk ketogenic response.

Sudden infant death has also been reported [9, 10]. One such infant had brick red urine, indicative of myoglobinuria. Autopsy revealed a fatty liver. Fulminant hepatic failure has also been observed, treated by liver transplantation [10].

Other patients have presented with a picture of hyperketotic hypoglycemia [10]. There may be vomiting,

dehydration, or lethargy; or onset may be with seizures. One patient had hyponatremia [10]. The liver may be enlarged.

Another presentation is with hyperinsulinemic hypoglycemia [11, 12]. In one Pakistani family in which parents were doubly heterozygous, hyperinsulinism required treatment with diazoxide [8]. Of four affected children, two died. One had impaired mental development and one had developed normally. An infant in another family had hyperinsulinism that was readily controlled with diazoxide and hydrochlorthiazide [11].

Laboratory evaluation has revealed hypoketosis in some patients [1, 10]. Analysis of the free fatty acids of the plasma or organic acid analysis of the urine may reveal medium chain 3-hydroxy fatty acids, even when the patient is metabolically well [12, 13]. However, this pattern may also be seen in infants receiving medium chain triglycerides.

The concentration of free-carnitine in the plasma may be normal or low, and there may be increased quantities of carnitine esters in the urine. Medium chain dicarboxylic aciduria is characteristic, but the levels are not high, and at times they may be normal. Adipic, suberic, and sebacic acids are found, as well as 3-hydroxydicarboxylic acids. In our patient, challenge with a load of medium-chain triglyceride led to increased excretion of dicarboxylic acids and 3-hydroxydicarboxylic acids. She always excreted elevated amounts of trans-cinnamoyl glycine.

The acylcarnitine profile reveals C₄-OH carnitine which could indicate the 3-hydroxybutyrylcarnitine of HADH disease, but this would not be different in the presence of 3-hydroxyisobutyrylCoA deacylase deficiency or D-3-hydroxybutyrylcarnitine in a ketone body utilization defect. In one patient [9], elevated C16 and C18 acylcarnitines were found, and no hydroxyacylcarnitines.

GENETICS AND PATHOGENESIS

The disorder is transmitted in an autosomal recessive fashion. Consanguinity has been observed and heterozygosity has been demonstrated by enzyme assay of the liver [9] and by mutational analysis [12].

The enzyme HADH is a soluble mitochondrial matrix enzyme with two identical subunits [3]. The gene encodes a 34-kDa precursor protein that, with processing, yields a mature 31-kDa subunit. Its substrate specificity is considerably broader than the name would suggest. It is most highly active against hydroxybutyryl CoA, but it is active up to C16. Deficiency of enzyme activity has been demonstrated in muscle [1], liver [9], and fibroblasts [12], as well as in mitochondria isolated from fibroblasts [11]. In some families, the defective enzyme was not demonstrable in fibroblasts, but was found in muscle [1] or liver [9].

The rat and human cDNAs have been sequenced [6, 7]. The human gene encodes a protein of 314 amino acids

and is expressed in liver, kidney, heart, and muscle. The gene contains eight exons. Compound heterozygosity for two mutations (A118G p.A28T and C171 A p.D45E) has been observed in a patient with hepatic failure. In an Indian patient with hyperinsulinemic hypoglycemia, heterozygosity was found for C773T resulting in p.P258L. In a consanguineous Pakistani family, a deletion was found that removed the acceptor splice site adjacent to exon 5 and led to deletion of exon 5 in the mRNA14.

TREATMENT

Treatment with carnitine and a diet low in fat appears to be prudent. The avoidance of fasting is important and supplemental cornstarch may be useful.

REFERENCES

1. Tein I, De Vivo DC, Hale DE *et al*. Short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency in muscle: a new cause for recurrent myoglobinuria and encephalopathy. *Ann Neurol* 1991; **30**: 415.
2. Haas RH, Marsden DL. Disorders of organic acids. In: Berg BO (ed.). *Principles of Child Neurology*. New York: McGraw-Hill, 1996: 1049.
3. Noyes BE, Bradshaw RA. L-3-Hydroxyacyl coenzyme A dehydrogenase from pig heart muscle. I. Purification and properties. *J Biol Chem* 1973; **248**: 3042.
4. He XY, Yang SY, Shultz H. Assay of L-3-hydroxyacyl-dehydrogenase with substrates of different chain lengths. *Anal Biochem* 1989; **180**: 105.
5. Uchida Y, Izai K, Orii T, Hashimoto T. Novel fatty acid β -oxidation enzymes in rat liver mitochondria. II. Purification and properties of enoyl-coenzyme A (CoA) hydratase/3-hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase trifunctional protein. *J Biol Chem* 1992; **267**: 1034.
6. Amaya Y, Takiguchi M, Hashimoto T, Mori M. Molecular cloning of cDNA for rat mitochondrial 3-hydroxyacyl-CoA dehydrogenase. *Eur J Biochem* 1986; **156**: 9.
7. Vredendaal PJ, van den Berg IE, Malingre HER *et al*. Human short-chain L-3-hydroxyacyl-CoA dehydrogenase: cloning and characterization of the coding sequence. *Biochem Biophys Res Commun* 1996; **223**: 718.
8. O'Brien LK, Rinaldo P, Sims HF *et al*. Fulminant hepatic failure associated with mutations in the medium and short chain L-3-hydroxyacyl-CoA dehydrogenase gene. *J Inher Metab Dis* 2000; **23**: 127 (Abstr.).
9. Treacy EP, Lambert DM, Barnes R *et al*. Short-chain hydroxyacyl-coenzyme A dehydrogenase deficiency presenting as unexpected infant death: a family history. *J Pediatr* 2000; **137**: 257.
10. Bennet MJ, Weinberger MJ, Kobori JA *et al*. Mitochondrial short-chain L-3-hydroxyacyl-coenzyme A dehydrogenase deficiency: a new defect of fatty acid oxidation. *Pediatr Res* 1996; **39**: 185.

11. Clayton PT, Eaton S, Aynsley-Green A *et al*. Hyperinsulinism in short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency reveals the importance of beta-oxidation in insulin secretion. *J Clin Invest* 2001; **108**: 457.
12. Sovik O, Matre G, Rishaug U *et al*. Familial hyperinsulinemic hypoglycemia with a mutation in the gene encoding short-chain 3-hydroxyacyl-CoA dehydrogenase. *J Inherit Metab Dis* 2002; **25**: 63.
13. Jones PM, Quinn R, Fennessey PV *et al*. Improved stable isotope dilution-gas chromatography-mass spectrometry method for serum or plasma free 3-hydroxy-fatty acids in its utility for the study of disorders of mitochondrial fatty acid beta-oxidation. *Clin Chem* 2000; **46**: 149.
14. Sim KG, Hammond J, Wilcken B. Strategies for the diagnosis of mitochondrial fatty acid β -oxidation disorders. *Clin Chim Acta* 2002; **323**: 37.
15. Molven A, Matre GE, Duran M *et al*. Familial hyperinsulinemic hypoglycemia caused by a defect in the SCHAD enzyme of mitochondrial fatty acid oxidation. *Diabetes* 2004; **53**: 221.

Short/branched chain acyl-CoA dehydrogenase (2-methylbutyrylCoA dehydrogenase) deficiency

Introduction	312	Treatment	314
Clinical manifestations	312	References	314
Genetics and pathogenesis	312		

MAJOR PHENOTYPIC EXPRESSION

Episodic lethargy, hypoglycemia, and acidosis; hypotonia; impaired mental development; possibly asymptomatic; 2-methylbutyrylglutininuria; 2-methylbutyrylcarnitinemia; and short/branched chain acyl-CoA dehydrogenase deficiency.

INTRODUCTION

Short/branched chain acyl-CoA dehydrogenase (SBCAD) deficiency may be an inborn error of metabolism, which does not manifest clinically unless the patient undergoes some level of metabolic stress. Of two affected siblings in the initial report of Gibson and colleagues in 2000 [1], the first manifested neurologic abnormalities following a probable ischemic/hypoxic event at 3 days of age. His sister, identified prenatally, had been completely asymptomatic by the time of follow-up report [2], and a number of other patients has been asymptomatic, particularly those identified by neonatal screening [2–5].

The key to the metabolic abnormality was the excretion of 2-methylbutyrylglutinine in the urine and an elevated level of 2-methylbutyrylcarnitine in the blood. The activity of 2-methylbutyryl CoA dehydrogenase (Figure 43.1) in fibroblasts was found to be deficient [3] and Western blot revealed absence of the enzyme in one family [1]. The cDNA for the SBCAD gene was isolated by Rozen *et al.* [6] and mapped to chromosome 10q25-q24 [7]. At least three mutations have been identified [1–3].

CLINICAL MANIFESTATIONS

The initial patient [1] was first admitted at 3 days of life with a life-threatening episode characterized by hypoglycemia, dehydration, lethargy, and hypothermia. Acidosis

was mild and there was no massive ketosis. Magnetic resonance imaging (MRI) revealed increased signal in the lentiform nuclei, and the electroencephalogram (EEG) was abnormal. By 12 months of age, he was behind in visual, motor, and cognitive skills and carried a diagnosis of athetoid cerebral palsy. 2-Methylbutyrylcarnitine was found in the blood and 2-methylbutyrylglutinine was found in the urine. In another family [2, 3], the patient was a three-year-old product of a consanguineous mating who had hypotonia and impaired motor development. MRI was normal. He had 2-methylbutyrylglutininuria, but a normal acylcarnitine profile. His asymptomatic mother also excreted 2-methylbutyrylglutinine. Among ethnic Hmongs, eight patients have been found on expanded newborn screening to have 2-methylbutyrylglutininuria and a distinct mutation [4]. Except for mild muscular hypotonia observed at six months of age, all were asymptomatic. Of two other consanguineous patients, one had attention-deficient disorder, and one convulsive disease and developmental delay. It appears that patients with this disorder may be asymptomatic, but neurological disease may be a feature.

GENETICS AND PATHOGENESIS

SBCAD deficiency is coded for by an autosomal recessive gene on chromosome 10 [6]. It was localized to 10q25-q26 by fluorescence *in situ* hybridization.

The gene contains 11 exons [7]; its open reading frame

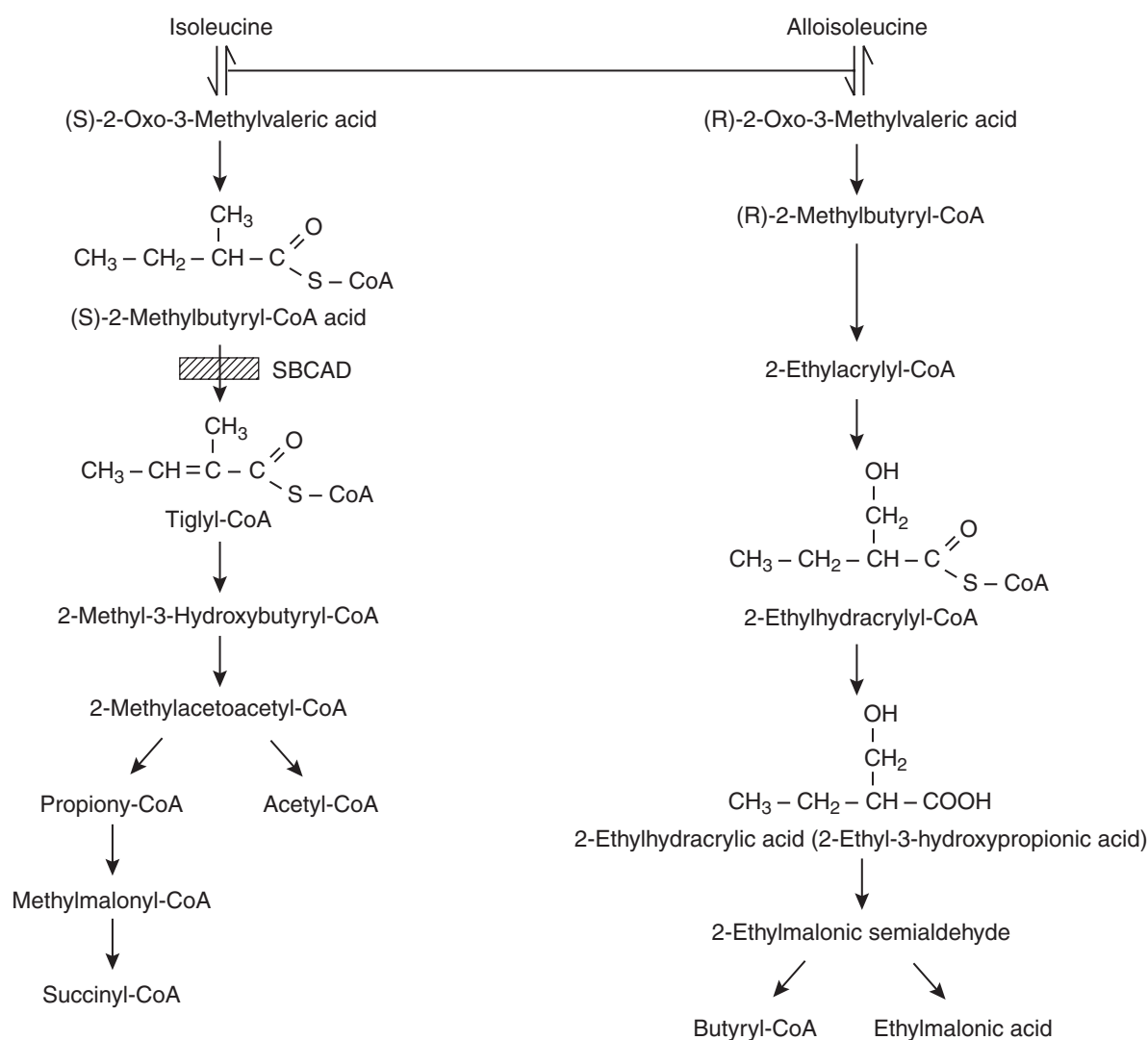


Figure 43.1 Metabolic pathways relevant to short/branched chain acyl-CoA dehydrogenase deficiency. Interrelations of the S and R pathways of isoleucine catabolism are shown.

of 1.3 kb encodes a precursor protein of 431 amino acids, which is processed to a mature protein of 399 amino acids. The SBCAD protein is imported into the mitochondria and forms a tetramer. The cDNA has considerable sequence homology with other acyl-CoA dehydrogenases (ACAD). Greatest homology is to short chain acyl-CoA dehydrogenase (SCAD) (Chapter 41) and ACAD-8 [8] which is an isobutyrylCoA dehydrogenase. The activity of SBCAD is greatest toward 2-methylbutyrylCoA, but it reacts with other 2-methylbranched chain substrates and with short chain acyl-CoA compounds, including butyryl-CoA. It has little or no activity against isobutyrylCoA.

Mutational analysis in the first patient revealed a C778T substitution in the coding region which led to the substitution of a phenylalanine for leucine at amino acid 22 [1]. Mutational analysis revealed homozygosity for a G1228A transition in the second patient and his mother

[3]. This led to skipping of exon 10 and a 100-bp deletion. The patient was also heterozygous for the common A625 variant SCAD allele. Mutational analysis in the Hmong patients yielded a homozygous A1165G mutation, which also led to skipping of exon 10. Four additional mutations identified [9] were C443T, A848G, T1102C, and G9085C. These mutations led to defective activity of the SBCAD enzyme.

In the first patient [1], the conversion of ^{14}C -isoleucine to $^{14}\text{CO}_2$ in intact fibroblasts was impaired. Incubation of ^{13}C -isoleucine with L-carnitine in intact cultured fibroblasts led to accumulation of isotope in C5-acylcarnitine. Western blot analysis revealed absence of the SBCAD protein. In the second patient [3], the activity of 2-methylbutyrylCoA dehydrogenase in fibroblasts was 10 percent of control. Defective activity was also demonstrated by expressing the abnormal gene products in *E. coli* and

COS cells [1–3]. In the Hmong patients, there was also no cross-reactive material (CRM) [4]. Prenatal diagnosis of an affected fetus has been accomplished [1].

Defective activity of SBCAD enzyme leads to accumulation of 2-methylbutyryl-CoA and its conjugation products 2-methylbutyrylglycine and 2-methylbutyryl (C5) carnitine. Tandem mass spectrometry (MS/MS) has been invaluable for the identification of this disorder of isoleucine metabolism. Many of those reported have been found through programs of expanded newborn screening. Elevated C5 acylcarnitine may be documented by analysis of the plasma, as well as of dried blood on filter paper. Plasma concentrations reported have varied from 0.7 to 3.4 $\mu\text{mol/L}$; controls were <0.6 [4]. In blood spots, levels ranging from 0.5 to 2.5 $\mu\text{mol/L}$ have been observed; reference <0.46 [5]. 2-Methylbutyrylglycinuria is identified by organic acid analysis of the urine. Levels reported have ranged from 3 to 73 mmol/mol creatinine [1–5, 9]. Normal levels are generally less than 2 mmol/mol creatinine. It is clear from the range observed that some patients display a peak that is so small it could be missed on organic analysis. Acylglycines are considered to be recognized with inadequate sensitivity by gas chromatography-mass spectrometry (GCMS) because of variable extraction, chromatographic instability, or failure of spectrum recognition [9]. This has led to methods of stable isotope dilution, selected ion monitoring GCMS [10] or MS/MS [11]. Patients with this disease have also been found to excrete 2-ethylhydracrylic (2-ethyl-3-hydroxypropionic) acid (Figure 43.1) [9] and this may serve as another recognition marker for organic acid analysis. The amounts of this compound usually exceed that of 2-methylbutyrylglycine. Quantification has not been perfect, for there is no commercial standard for 2-ethylhydracrylic acid, but comparison arbitrary units ranged from 8 to 152 in four patients (controls <5) [9]. Chiral determination of 2-methylbutyric acid indicated that 40–46 percent was in the form of the R isomer in patients and in controls. There are two pathways of isoleucine catabolism (Figure 43.1). Isoleucine itself is predominately in S chiral form. It is transaminated to its keto acid, 2-oxo-3-methylvaleric acid, which is subsequently oxidized via the S pathway to (S)-2-methylbutyrylCoA where the next step is catalyzed by the SBCAD enzyme [5]. Keto-enol tautomeric racemization following, or enamine tautomerization during, transamination is the source of alloisoleucine (Chapter 19). 2-Ethylhydracrylic acid is on the R pathway. Its occurrence in SBCAD deficiency indicates that this enzyme does not catalyze the conversion of R-2 methylbutyryl-CoA to 2-methylacrylylCoA.

Ethylhydracrylic acid excretion in increased quantity may also be observed in ketosis [12], in 3-oxothiolase deficiency [13], in 2-methyl-3-hydroxybutyrylCoA dehydrogenase deficiency [14], in propionic acidemia [15], and methylmalonic acidemia, all defects in steps of the S pathway. It may also be found in ethylmalonic

encephalopathy (Chapter 107), hydroxyisobutyric aciduria, and in Barth syndrome.

Prenatal diagnosis was accomplished [1] by analysis of 2-methylbutyrylglycine in amniotic fluid (0.27 $\mu\text{mol/L}$, normal <0.03) and of C5-acylcarnitine (1.93 $\mu\text{mol/L}$; normal 0.37 ± 0.18) [14].

TREATMENT

Patients have been treated with carnitine in doses of 50–100 mg/kg per day and diets restricted in protein or isoleucine [2, 4], but poor compliance and discontinuation have occurred without obvious clinical consequences [4]. A protein intake of 1–4 g/kg along with carnitine of 71 mg/kg led to a normal excretion of 2-methylbutyrylglycine [1].

REFERENCES

- Gibson KM, Burlingame TG, Hogema B *et al*. 2-Methylbutyryl-coenzyme A dehydrogenase deficiency: a new inborn error of L-isoleucine metabolism. *Pediatr Res* 2000; **47**: 830.
- Akaboshi S, Ruiters J, Wanders RJA. Divergent phenotypes in siblings with confirmed 2-methylbutyryl-CoA dehydrogenase (2-MBCD) deficiency. *J Inher Metab Dis* 2001; **24**(Suppl. 1): 58.
- Andresen BS, Christensen E, Corydon TJ *et al*. Isolated 2-methylbutyrylglycinuria caused by short/branched-chain acyl-CoA dehydrogenase deficiency: identification of a new enzyme defect, resolution of its molecular basis, and evidence for distinct acyl-CoA dehydrogenases in isoleucine and valine metabolism. *Am J Hum Genet* 2000; **67**: 1095.
- Matern D, He M, Berry SA *et al*. Prospective diagnosis of 2-methylbutyryl-CoA dehydrogenase deficiency in the Hmong population by newborn screening using tandem mass spectrometry. *Pediatrics* 2003; **112**: 74.
- Korman SH, Barash V, Corydon TJ *et al*. Short/branched-chain acyl-CoA dehydrogenase (SBCAD) deficiency; expanded clinical and molecular spectrum. *J Inher Metab Dis* 2001; **24**(Suppl. 1): 68 (Abstr.).
- Rozen R, Vockley J, Zhou L *et al*. Isolation and expression of a cDNA encoding the precursor for a novel member (ACADSB) of the acyl-CoA dehydrogenase gene family. *Genomics* 1994; **24**: 280.
- Arden KC, Viars CS, Fu K *et al*. Localization of short/branched chain acyl-CoA dehydrogenase (ACADSB) to human chromosome 10. *Genomics* 1995; **25**: 743.
- Telford EA, Moynihan LM, Markham AF *et al*. Isolation and characterization of a cDNA encoding the precursor for a novel member of the acyl-CoA dehydrogenase gene family. *Biochim Biophys Acta* 1999; **1446**: 371.
- Korman SH, Andresen BS, Zeharia A *et al*. 2-Ethylhydracrylic aciduria in short/branched-chain acyl-CoA dehydrogenase deficiency: application to diagnosis and implications for the R-pathway of isoleucine oxidation. *Clin Chem* 2005; **51**: 610.

10. Rinaldo P, O'Shea JJ, Welch RD *et al.* Stable isotope dilution analysis of n-hexanoylglycine, 3-phenylpropionylglycine and suberylglycine in human urine using chemical ionization gas chromatography/mass spectrometry selected ion monitoring. *Biomed Environ Mass Spectrom* 1989; **18**: 471.
11. Bonafe L, Troxler H, Kuster T *et al.* Evaluation of urinary acylglycines by electrospray tandem mass spectrometry in mitochondrial energy metabolism defects and organic acidurias. *Mol Genet Metab* 2000; **69**: 302.
12. Liebich HM, Forst C. Hydroxycarboxylic and oxocarboxylic acids in urine: products from branched-chain amino acid degradation and from ketogenesis. *J Chromatogr* 1984; **309**: 225.
13. Mamer OA, Tjoa SS. 2-Ethylhydracrylic acid: a newly described urinary organic acid. *Clin Chim Acta* 1974; **55**: 199.
14. Ensenauer R, Niederhoff H, Rüter JP *et al.* Clinical variability in 3-hydroxy-2-methylbutyryl-CoA dehydrogenase deficiency. *Ann Neurol* 2002; **51**: 656.
15. Przyrembel H, Bremer HJ, Duran M *et al.* Propionyl-CoA carboxylase deficiency with overflow of metabolites of isoleucine catabolism at all levels. *Eur J Pediatr* 1979; **130**: 1.

Multiple acyl CoA dehydrogenase deficiency/ glutaric aciduria type II/ethylmalonic-adipic aciduria

Introduction	316	Treatment	321
Clinical abnormalities	317	References	322
Genetics and pathogenesis	320		

MAJOR PHENOTYPIC EXPRESSION

Overwhelming neonatal illness with metabolic acidosis, acrid odor, hypoketotic hypoglycemia, and hyperammonemia; dysmorphic features; polycystic kidneys; massive urinary excretion of lactic and glutaric acids, and increased concentrations of many other organic acids, including ethylmalonic acid, butyric acid, methylbutyric acid, isobutyric and isovaleric acids, and deficiency of electron transfer flavoprotein (ETF) or its dehydrogenase (ETF-QO). Later onset, milder variants referred to as ‘ethylmalonic-adipic aciduria’, may first present in the neonatal period or adulthood with episodic illness characterized by vomiting, hypoglycemia, and lipid storage myopathy.

INTRODUCTION

Glutaric aciduria type II was first reported in 1976 by Przyrembel *et al.* [1] in an infant with severe hypoglycemia and profound metabolic acidosis without ketosis. Patients with this form of the disorder have overwhelming illness in the neonatal period that has been uniformly fatal. The name was employed to distinguish the disease from the glutaric aciduria due to defective activity of glutaryl CoA dehydrogenase ([Chapter 8](#)) that had been reported a year earlier by Goodman and colleagues [2]. Organic acid analysis revealed the accumulation of a wide variety of organic acids, including lactic, isovaleric, and ethylmalonic

acids, as well as glutaric acid. There is generalized defect in the activity of many acyl CoA dehydrogenases [3]. Thus, the term ‘multiple acyl CoA dehydrogenase deficiency’ (MADD) is more descriptive, it has variously been abbreviated MAD deficiency and MADD; it has also been divided into severe (MAD:S) and mild (MAD:M) forms [4], but there is sufficient heterogeneity of clinical expression that these are not useful.

The fundamental molecular defect is in the mitochondrial transport of electrons from the acylCoAs to ubiquinone (CoQ10) of the main electron transport chain [5–7]. The defect may be in any of three proteins, the alpha or beta subunits of electron transfer flavoprotein (ETF)

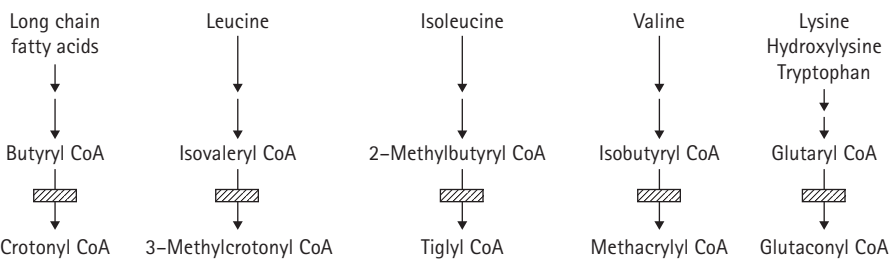


Figure 44.1 Multiple acyl CoA dehydrogenase deficiency involves the dehydrogenation of many CoA intermediates in fatty acid and amino acid catabolism.

or its dehydrogenase, ETF-ubiquinone oxidoreductase (ETF-QO) (EC 1.5.5.1). Both are flavoproteins. Another designation has been IIA and IIB for defects in the α and β proteins and IIC for ETF-QO defects. Patients with congenital anomalies have defects in ETF-QO.

The mitochondrial oxidations of glutaryl CoA and other intermediates in branched-chain amino acid metabolism, and the β -oxidation of fatty acids (Figure 44.1) are catalyzed by mitochondrial flavinadenine dinucleotide (FAD)-dependent enzymes [8–11]. Each of the dehydrogenase enzymes of fatty acid oxidation, and the amino acid catabolic enzymes catalyze the dehydrogenation of saturated acylCoA compounds to form the 2,3-unsaturated or enoylCoA thioesters (Figure 44.2). Both sarcosine and dimethylglycine are catabolized by specific N-methyldehydrogenases containing covalently bound FAD and dissociable folic acid cofactors [12–14], and thus these two compounds may also accumulate in this disease. Each dehydrogenase enzyme contains a molecule of FAD.

ETF is a mitochondrial matrix heterodimer containing an AMP molecule and a single noncovalently bound FAD which accepts hydrogens from all of the acylCoA dehydrogenases [15–19]. ETF has a 30-kD α - and a 25-kD β -subunit. ETF-QO is a 64-kD monomer iron-sulfur-containing ($^4\text{Fe-}^4\text{S}$) flavoprotein (previously referred to as Fe-S flavoprotein) that accepts electrons from reduced ETF and transmits them to coenzyme Q and the cytochrome chain (Figure 44.2) [7, 20–23].

The cDNAs for the α - and β -subunits of ETF [24, 25] and ETF-QO [26] have been cloned and sequenced. α -ETF and ETF-QO have N-terminal mitochondrial import sequences, but the β -subunit cDNA does not encode a leader peptide, and so does not undergo such processing. The gene for α -ETF has been localized to chromosome 15 at q23–25 [27], that of β -ETF on chromosome 19 [28], and that for ETF-QO on chromosome 4 [29] at q32–qter. Mutations have been identified in α -ETF [30, 31], the most common of which appears to be a change at codon 266 from threonine to methionine. In ETF-QO, a number of apparently rare mutations have been identified which lead to an absence of enzyme activity and immunoreactive proteins [32].

CLINICAL ABNORMALITIES

The infant with classic multiple acyl CoA dehydrogenase deficiency presents with life-threatening illness in the first

days of life. The clinical picture is reminiscent of those of the typical organic acidemias, propionic acidemia (Chapter 2), methylmalonic acidemia, (Chapter 3) and isovaleric acidemia (Chapter 7), but the severity of illness in this disease is so great that all three of the patients we have studied died after less than 90 hours of life [33, 34], and most of those reported have died within the first week [1, 35–41].

These infants [1, 33] develop tachypnea or dyspnea within a few hours of birth. They are found to have profound metabolic acidosis and impressive hypoglycemia. In spite of intravenous glucose and NaHCO_3 , the concentration of glucose in the blood may decrease as does the pH, and cardiac arrest soon follows, and despite resuscitation and artificial ventilation, the course is inexorable.

The first patient was described as having a ‘very disagreeable sweaty-feet odor’ [1]. We described our first patient [33] as having a peculiar, acrid odor. This is the consequence of an excess of a number of short chain, volatile organic acids. A number of these patients has been described as pale [1, 33, 34] and one had macrocytic anemia and a hemoglobin concentration of 9.1 g/dL. Many have had convulsions consistent with the degree of depression of the blood glucose. Hyperammonemia was a consistent feature in our patients [33, 34]. Fatty infiltration of the liver is found postmortem.

A number of the neonatal onset patients have had prominent dysmorphic features (Figures 44.3, 44.4, 44.5, 44.6, 44.7, and 44.8) [33, 34, 42]. They include a high forehead, depressed nasal bridge, and a short anteverted nose. The ears may be low-set, malrotated, and abnormally formed (Figures 44.4 and 44.5). Muscular defects of the abdominal wall have occurred, as well as genital defects, such as hypospadias and chordee. Some have had macrocephaly [43]. Minor anomalies include horizontal palmar creases and rocker-bottom feet (Figure 44.6). One of our patients also had an interventricular septal defect and three umbilical vessels. Some patients [33] have been described as premature or small for gestational age.

A major malformation in these infants is the occurrence of polycystic kidneys. The kidneys may be huge and readily palpable. Abnormally small prenatal production of urine may be the cause of the semilunar folds below the eyes, as in the Potter syndrome (Figure 44.3), and typical Potter syndrome, including pulmonary hypoplasia has been observed [36]. Polycystic kidneys may be present in infants without dysmorphic features and may be found first at

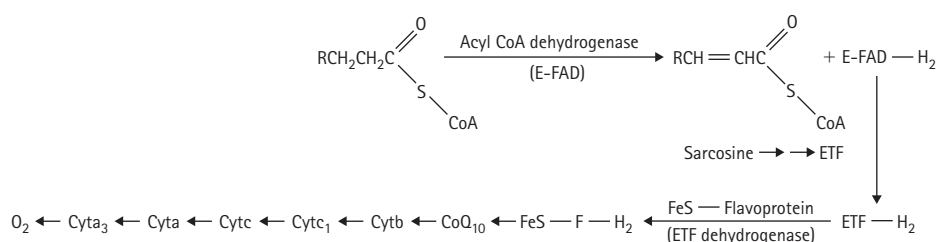


Figure 44.2 The roles of electron transfer flavoprotein (ETF) and ETF-QO oxidation and the passage of electrons along the electron transport chain.



Figure 44.3 Postmortem pictures of Baby M [32, 33] who died of glutaric aciduria type II on the first day of life. He had a low, incompletely rotated ear with a reduced anthelix, and he had three umbilical vessels. Autopsy revealed large polycystic kidneys and an interventricular septal defect.



Figure 44.5 The right ear was also abnormal in position and appearance.



Figure 44.4 Baby M had a high forehead, depressed nasal bridge, short nose with anteverted nares, and a long philtrum. The ears were low-set and he had semilunar folds below the eyes.



Figure 44.6 The hand was short and broad, and had a single horizontal crease.



Figure 44.7 Baby girl N died of intractable acidosis in the first week. She had enormous polycystic kidneys.



Figure 44.8 Baby girl N was not strikingly dysmorphic. She did have anteverted nares and a triangular-shaped mouth. The ears appeared low.

autopsy [37, 38, 42]. Ultrastructural changes have been described in the glomerular basement membrane [43]. Other pathologic abnormalities include cerebral gliosis and heterotopias giving a warty dysplastic appearance to the cortex [36]. Electron dense membrane limited bodies have been reported in the brain and kidneys [41]. Hepatic periportal necrosis has been reported [43], and more commonly hepatic microvesicular lipid. Pancreatic ducts may be hypoplastic [36].

Infants without dysmorphic features and abnormal organogenesis have also presented early in life with tachypnea, acidosis, hypoglycemia, and an abnormal odor. Many have had hepatomegaly. Some of these have survived the initial episode and died a few months later, often with cardiomyopathy. A small number has survived a bit longer and had episodic illness reminiscent of Reye syndrome [44–47]. Sudden life-threatening disease in infancy was reported [48] in three infants with this disease, two of whom died. One had documented arrhythmia and the authors attributed the episode to myocardial dysfunction in the others.

The later onset multiple acyl CoA dehydrogenase deficiency, or ethylmalonic-adipic aciduria has presented with a considerable variety. The first patient reported [49] had episodic vomiting, hypoglycemia, and acidosis from 7 weeks of age. Another [50] presented first as a 19-year-old in hypoglycemic coma and continued to have episodes of nausea, vomiting, hypoketotic hypoglycemia, and hepatic dysfunction with elevated bilirubin and transaminases, but normal ammonia. Two sisters had died in childhood of the same disease. Others have had such episodes beginning in the first year of life [51–53], but one woman presented at 25 years with a history of episodic muscle weakness and vomiting [54].

An adult patient [50] had muscle weakness and low levels of carnitine in muscle. Others had lipid storage myopathy and systemic deficiency of carnitine [55–57]. During acute episodes, these patients have had hypoketotic

hypoglycemia, acidosis and sometimes, especially early in life, hyperammonemia. Transaminase levels in blood may be elevated, and there may be prolongation of prothrombin or partial thromboplastin times. Lactic acidemia may be impressive. In Taiwanese patients with mutations in the *ETFDH* gene, the phenotype was myopathic but highly variable [57]. At one extreme, a ten-year-old girl developed progressive weakness of proximal muscles and died of cardiopulmonary failure, acidosis, hypoglycemia, and hyperammonemia; on the other hand, a 27-year-old woman with exercise intolerance since childhood had episodes of pancreatitis, elevated creatine kinase, and lipid droplets in biopsied muscle. Her older sister had an even milder phenotype. Both responded well to riboflavine and carnitine.

Roentgenograms of the chest may reveal cardiomegaly, and echocardiography may be consistent with cardiomyopathy. Neuroimaging may reveal areas of increased signal on T_2 of the magnetic resonance image (MRI) in the basal ganglia (Figure 44.9) [58] or hypomyelination [59]. A macrocephalic patient was found to have symmetric hypoplasia of the temporal lobes of the brain in the first week of life [59]. He had normal psychomotor development for 11 months when he died of a sudden cardiac arrest. Autopsy showed hypomyelination and systemic hypoplasia of temporal lobes with loss of axons and focal subcortical ganglionic heterotopia, consistent with aberrant intrauterine developmental origin.

The diagnosis in all forms of multiple acylCoA dehydrogenase deficiency has usually been made on the basis of the unusual pattern of organic acid excretion in which a large

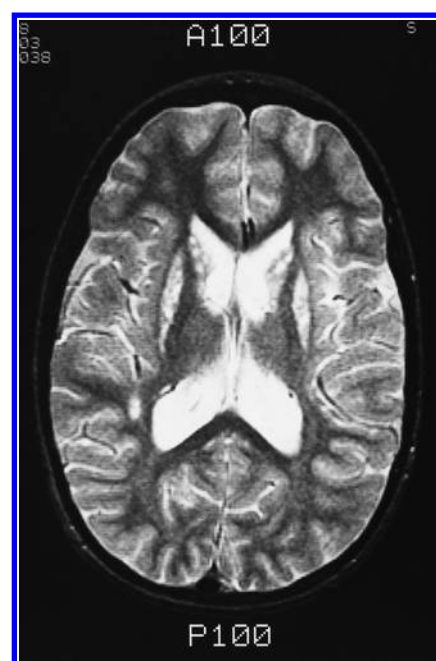


Figure 44.9 MH: Neuroimaging (magnetic resonance imaging) of the brain reveals an extraordinary pattern of increased T_2 signal in the white matter.

number of organic acids are found in elevated amount in the urine. This is especially true in the severe neonatal onset form in which the quantities found are enormous. The most prominent of these are lactic acid and glutaric acid, but a large number of other dicarboxylic acids and hydroxy acids are found. Among the former are ethylmalonic, adipic, suberic, and sebacic acids, as well as unsaturated suberic acids. Among the latter are 2-hydroxybutyric, 2-hydroxyglutaric, and 5-hydroxyhexanoic acids. 3-Hydroxyisovaleric and 2-hydroxyisocaproic acids are also found in the urine. Most of the same organic acids are found in increased amounts in the plasma. The concentrations of glutaric and lactic acids are prominent. p-Hydroxyphenyllactic acid may be elevated in the blood and the urine, possibly an index of immaturity or of hepatic disease.

Volatile acids are demonstrable in the plasma by analysis with gas liquid chromatography. The concentrations of isovaleric, acetic, isobutyric, 2-methylbutyric, butyric, and propionic acids may all be elevated to values 60–4800 times normal. In our first patient, the concentration of isovaleric acid was 0.76 mmol/L [33]. This would account for the odor. Elevated concentrations of these compounds are also found in the urine. Isovaleryl glycine is found in the urine, as is N-isovaleryl glutamic acid [46]. Acylcarnitine profiles reveal multiple esters of organic acids (Figure 44.10).

The organic aciduria is not nearly so pronounced in the milder or episodic forms of the disease. Some only manifest increased excretions of ethylmalonic and adipic acids [49]. In others, abnormal quantities of organic acids are found only during acute episodes of illness. The excretion of 2-hydroxyglutaric acid is a useful marker for this disease. This contrasts with glutaryl-CoA dehydrogenase deficiency (Chapter 8) in which 3-hydroxyglutaric acid is the hydroxy acid found.

In the neonatal onset disease, the concentrations of the amino acids, citrulline, lysine, ornithine, and proline are elevated in plasma and urine. Hydroxyproline excretion may be high, consistent with a generalized amino aciduria. The excretion of arginine may also be very high. In the later onset disease, there may be elevated concentrations of sarcosine in blood and urine [36, 40, 44]. Concentrations of carnitine in the blood may be low [57].

GENETICS AND PATHOGENESIS

The disease in each of its forms is autosomal recessive. Intermediate activities of enzymes have been documented in parents of a patient in whose fibroblasts ETF-QO was deficient [6, 7] and in parents of a patient with a mild variant [49].

Prenatal diagnosis has been accomplished by the demonstration of large amounts of glutaric acid in amniotic fluid [60, 61]. It has also been done by documenting impaired oxidation of [46, 47, 62] substrate and by immunochemical assay [63] in cultured amniocytes.

In addition to clinical heterogeneity, heterogeneity has been observed in differing amounts of ETF and ETF-QO activity and antigen in different cell lines [64, 65]. In some, this has correlated with clinical severity [6], but in others it has not. Deficiency of ETF-QO has been found in the patients with anomalies and polycystic kidneys [40]. Deficiency of ETF-QO antigen was first demonstrated [40] in liver mitochondria of such an infant; it was also demonstrated in fibroblasts of this patient [7], and two others with renal cysts [66]. ETF-QO was nearly completely deficient in these patients, while the deficiency was less severe in patients with the later onset variants [6, 57, 67].

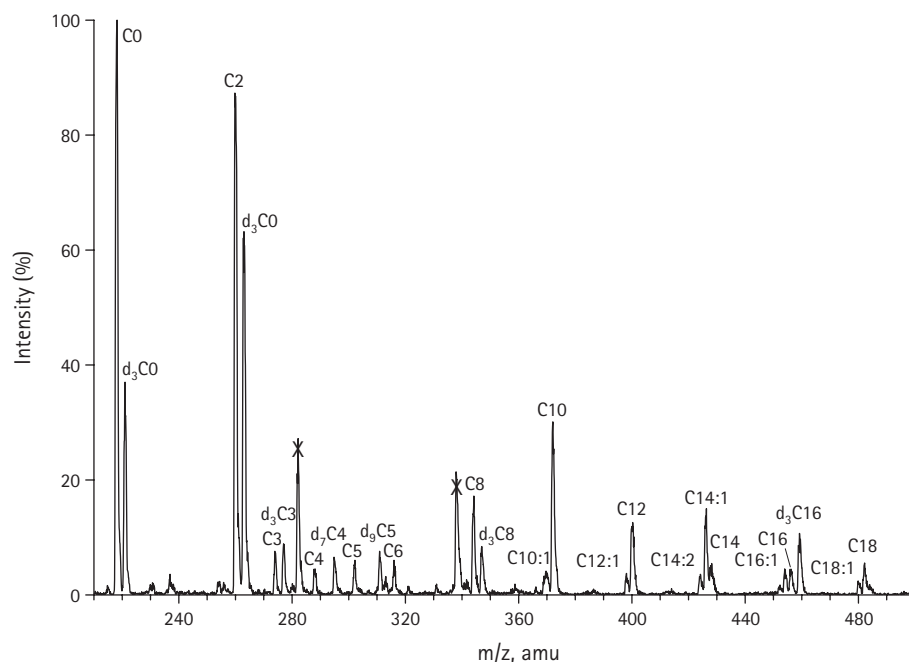


Figure 44.10 Acylcarnitine profile of the blood plasma of a patient with multiple acyl CoA dehydrogenase deficiency. The pattern of elevation of many acylcarnitine esters follows a curve with the maximum at C10. The two peaks marked X were artifacts. The elevated C0 indicated treatment with carnitine. (Illustration provided by Jon Gangoiti of University of California, San Diego.)

ETF deficiency was found by immunoblot analysis in fibroblasts of two neonatal patients with no congenital abnormalities [7]. In one, the α - and β -subunits were both deficient, and the α -subunit was of smaller size. In the other patient, the α -subunit was also small, while the β -subunit was of normal size. The biosynthesis of the α -subunit precursor in the first cell line was virtually absent; in the second an α -subunit was made that was about 1 kDa smaller than usual. In another patient with severe disease and no anomalies, there were two weak bands of α -ETF, one smaller than normal [68]. ETF activity was virtually completely absent in these cells and in another severely ill newborn; some residual activity was found in a few patients with milder disease [5, 6]. In three patients, β -ETF deficiency was found by immunochemical assay [6, 69]. In cell lines of some patients with multiple acyl CoA dehydrogenase deficiency with ETF and ETF-QO, activity was normal [70], raising the possibility of fundamental defects not yet discovered.

Complementation studies clearly distinguished cells of this disease from those of isovaleric acidemia [71] and provided evidence of two groups of patients with severe multiple acylCoA dehydrogenase deficiency.

Molecular analysis of the coding sequence of six patients with neonatal onset multiple acylCoA dehydrogenase deficiency revealed seven different mutations in the α -subunit of ETF [30, 72]. The most common was a substitution of methionine for threonine at codon 266, which was found in four unrelated patients. A valine 157 to glycine change has also been reported in two patients [30, 31], and a glycine 116 to arginine [30]. Three deletions were observed [30], as well as a deletion of the consensus G 5-prime splice site donor leading to an 81-bp deletion and a 26 amino acid deletion were found [73] in the late onset form (D128N).

In ETF- β mutations were found [73] in the late onset form (D128N). In Japanese brothers, compound heterozygosity was found for an arginine to glutamine change at 164 and a G to C change at the first nucleotide of the intron donor site leading to a deletion of 53 amino acids [74].

In the ETF-QO, a mutation changing a cysteine 561 to alanine led to a truncated protein [32]. An A84T mutation was found in four Taiwanese families [57]. In an infant with the neonatal onset phenotype with congenital anomalies, a homozygous 1-bp deletion of 36A was found which led to a frame shift at alanine 12 and a stop codon at amino acid 19 [73].

In the presence of defective activity of ETF or ETF-QO, the activities of a number of dehydrogenases are impaired. This has most commonly been demonstrated by the incubation of fibroblasts derived from the patient with ^{14}C -labeled substrates and measuring their conversion to $^{14}\text{CO}_2$. Substrates used have included labeled glutaric acid, valine, leucine, isoleucine, 2-oxoisovaleric acid, and 2-oxoisocaproic acid [1], as well as labeled lysine, palmitic acid, butanoic acid, and butyric acid.

The conversion of 1,5- ^{14}C -glutaryl CoA to $^{14}\text{CO}_2$ has been assessed in the absence of artificial electron acceptor as an assay for the presence of active ETF and ETF-QO [75, 76]. Assay for tritium release from ^3H -labeled palmitic acid is also deficient in fibroblasts of patients, and this assay has been employed in complementation studies [77].

Riboflavin responsiveness has been demonstrated by the study of the oxidation of ^{14}C -labeled substrates in fibroblasts cultured in the presence and absence of riboflavin supplemented media [3]. Defective oxidation was restored to normal levels by growth in riboflavin. After growth in riboflavin-depleted medium, the level of the patient's ETF activity fell to 59 percent of control, as did the level of ^{14}C -FAD-labeled by growth in ^{14}C -riboflavin. This is consistent with evidence of clinical and biochemical responsiveness to riboflavin [78] in the patient whose cells were studied.

Among the consequences of multiple acylCoA dehydrogenase deficiency is depletion of body stores of carnitine. In a later-onset patient with even relatively mild disease, carnitine deficiency may be expected. An adult-onset patient was reported to have low levels of free-carnitine in liver and muscle [50]. In a neonatal-onset patient, free-carnitine levels in the blood may be low, but they may be normal; however, carnitine esters in the urine are high [79, 80]. This excretion of esters is increased after treatment with carnitine [39]. Specific carnitine esters identified include acetylcarnitine, isobutyrylcarnitine, isovalerylcarnitine, hexanoylcarnitine, propionylcarnitine, and butyrylcarnitine. Rapid diagnosis may be made by the analysis of acylcarnitine profiles in plasma or blood spots on filter paper [81, 82], and the disease is detectable in programs of neonatal screening. In this disease, there is a general accumulation of acylcarnitines from C4 to C18 [81]. It has also been documented that this approach to diagnosis may miss a patient with mild MAD deficiency [81].

TREATMENT

The treatment of the neonatal-onset patient is supportive, especially the treatment of acidosis, hypoglycemia, and dehydration with huge amounts of appropriate fluids. Nevertheless, most of those with polycystic kidneys die promptly.

Later-onset patients, and those who survive the initial episode, should be assessed for riboflavin responsiveness, reported to be best judged by changes in the dicarboxylic aciduria. Most patients are treated with riboflavin in doses of 100–300 mg/24 hours [52, 53, 78, 83], as well as carnitine. A riboflavin-responsive boy was reported to develop progressive spasticity, ataxia, and leukodystrophy without ever experiencing acute metabolic imbalance [84]. Improvement after treatment with riboflavin and carnitine has been reported in patients with mutations in the *ETFDH* gene [57]. Restriction of the intake of fat

and protein may be prudent, dependent on the severity of the disease. Glycine supplementation may also remove accumulated CoA esters as their glycine conjugates, as in isovaleric acidemia (Chapter 7). In a nine-year-old patient with milder disease, glycine supplementation was as effective as carnitine supplementation in handling a medium-chain triglyceride (MCT) load [85]. Inasmuch as the major conjugated compounds excreted after glycine are different from those after carnitine, it would be prudent to treat patients with both.

Differential diagnosis: riboflavin transporter defect

Three patients were reported [86] who had progressive muscle weakness and paralysis of the diaphragm in whom patterns of acylcarnitine profiles and urinary organic acids suggested an attenuated form of multiple acylCoA dehydrogenase deficiency. They were found to be deficient in riboflavin and also of flavinmononucleotide and FAD. Levels of riboflavin were restored by treatment with riboflavin and clinical manifestation improved markedly. Mutations were found in the *C20orf54* gene which encodes the human homolog of the rat transporter for riboflavin. The first two patients were homozygous c.1198-2A>C, an acceptor splice site mutation; the other patient was heterozygous for p.W17R and p.Y213X. Mutations in this gene were independently found [87] in patients with the Brown–Vialletto–Van Laere syndrome (MIM 211530).

REFERENCES

1. Przyrembel H, Wendel U, Becker K *et al.* Glutaric aciduria type II: report of a previously underdescribed metabolic disorder. *Clin Chim Acta* 1976; **66**: 22.
2. Goodman SI, Markey SP, Moe PG *et al.* Glutaric aciduria: a 'new' disorder of amino acid metabolism. *Biochem Med* 1975; **12**: 12.
3. Gregersen N. The acyl-CoA dehydrogenation deficiencies. *Scand J Clin Invest* 1985; **45**: 1.
4. Rhead W, Roettger V, Marshall T, Amendt B. Multiple acyl-Coenzyme A dehydrogenation disorder responsive to riboflavin: Substrate oxidation flavin metabolism and flavoenzyme activities in fibroblasts. *Pediatr Res* 1993; **33**: 129.
5. Amendt BA, Rhead WJ. The multiple acyl-coenzyme A dehydrogenation disorders glutaric aciduria type II and ethylmalonic-adipic aciduria: mitochondrial fatty acid oxidation acyl-coenzyme A dehydrogenase and electron transfer flavoprotein activities in fibroblasts. *J Clin Invest* 1986; **78**: 205.
6. Loehr JP, Goodman SI, Frerman FE. Glutaric acidemia type II: heterogeneity of clinical and biochemical phenotypes. *Pediatr Res* 1990; **27**: 311.
7. Frerman FE, Goodman SI. Deficiency of electron transfer flavoprotein or electron transfer flavoprotein ubiquinone oxido-reductase in glutaric acidemia type II fibroblasts. *Proc Natl Acad Sci USA* 1985; **82**: 4517.
8. Besrat A, Polan CE, Henderson LM. Mammalian metabolism of glutaric acid. *J Biol Chem* 1969; **244**: 1461.
9. Hall C. Acyl CoA Dehydrogenase and electron transferring flavoprotein. In: Fleisher S, Packer L (eds). *Methods in Enzymology*, vol. 53. New York: Academic Press, 1978: 502.
10. Aberhart DJ, Tann CH. Substrate stereochemistry of isovaleryl CoA dehydrogenase: elimination of the 2-pre-R hydrogen in biotin-deficient rats. *Bioorg Chem* 1981; **10**: 200.
11. Ikeda Y, Tanaka K. 2-Methyl-branched chain acyl-CoA dehydrogenase from rat liver. *Method Enzymol* 1988; **166**: 360.
12. Beinert H, Frisell WR. The functional identity of the electron-transferring flavoproteins of the fatty acyl coenzyme A and sarcosine dehydrogenase systems. *J Biol Chem* 1962; **237**: 2988.
13. Frisell WR, Mackenzie CG. Separation and purification of sarcosine dehydrogenase and dimethylglycine dehydrogenase. *J Biol Chem* 1962; **237**: 94.
14. Wittwer AJ, Wagner C. Identification of the folate-binding proteins of rat liver mitochondria as dimethylglycine dehydrogenase and sarcosine dehydrogenase flavoprotein nature and enzymatic properties of the purified proteins. *J Biol Chem* 1981; **256**: 4109.
15. Hoskins DD, Bjur RA. The oxidation of N-methylglycines by primate liver mitochondria. *J Biol Chem* 1964; **239**: 1856.
16. Hoskins DD, Bjur RA. The electron transferring flavoprotein of primate liver mitochondria. *J Biol Chem* 1965; **240**: 2201.
17. Hoskins DD. The electron transferring flavoprotein as a common intermediate in the mitochondrial oxidation of butyryl CoA and sarcosine. *J Biol Chem* 1966; **241**: 4471.
18. Husain M, Steenkamp DJ. Electron transfer flavoprotein from pig liver mitochondria. A simple purification and re-evaluation of some of the molecular properties. *Biochem J* 1983; **209**: 541.
19. McKean MC, Beckmann JD, Frerman FE. Subunit structure of electron transfer flavoprotein. *J Biol Chem* 1983; **258**: 1866.
20. Ruzicka FJ, Beinert H. A new membrane iron-sulfur flavoprotein of the mitochondrial transfer system. The entrance point of the fatty acyl dehydrogenation pathway. *Biochem Biophys Res Commun* 1975; **66**: 622.
21. Ruzicka FJ, Beinert H. A new iron-sulfur flavoprotein of the respiratory chain A component of the fatty acid β -oxidation pathway. *J Biol Chem* 1977; **252**: 8440.
22. Steenkamp DJ, Ramsay RR, Husain M. Reactions of electron transfer flavoprotein and electron transfer flavoprotein: ubiquinone oxidoreductase. *Biochem J* 1987; **241**: 883.
23. Frerman FE, Goodman SI. Fluorometric assay of acyl CoA dehydrogenases in normal and mutant fibroblasts lines. *Biochem Med* 1985; **33**: 38.
24. Finocchiaro G, Ito M, Ikeda Y, Tanaka K. Molecular cloning and nucleotide sequence of the cDNAs encoding the α -subunit of human electron transfer flavoprotein. *J Biol Chem* 1988; **263**: 15773.
25. Finocchiaro G, Colombo I, Garavaglia B *et al.* cDNA cloning and mitochondrial import of the β -subunit of the human electron-transfer flavoprotein. *Eur J Biochem* 1993; **213**: 1003.

26. Goodman SI, Bemelen KF, Frerman FE. Human cDNA encoding ETF dehydrogenase (ETF:ubiquinone oxidoreductase) and mutations in glutaric acidemia type II. In: Coates PM, Tanaka K (eds). *New Developments in Fatty Acid Oxidation*. New York: John Wiley & Sons, 1992: 567.
27. Barton DE, Yang-Feng TL, Finocchiaro G et al. Short chain acyl-CoA dehydrogenase (ACADS) maps to chromosome 12 (q22-ter) and electron transfer flavoprotein (ETFa) to 15 (q23-q25). *Cytogenet Cell Genet* 1987; **46**: 577.
28. Royal V, Alberts MJ, Pericak-Vance MA et al. RsaI RFLP for electron transfer flavoprotein-beta (ETFb). *Nucleic Acids Res* 1991; **19**: 4021.
29. Frerman FE, Goodman SI. Nuclear-encoded defects of the mitochondrial respiratory chain including glutaric acidemia type II. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic and Molecular Bases of Inherited Disease*. New York: McGraw-Hill, 1995: 1611.
30. Freneau E, Sheffield VC, Molin L et al. Glutaric aciduria type II: heterogeneity in the beta-oxidation flux polypeptide synthesis and complementary DNA mutations in the alpha subunit of electron transfer flavoprotein in eight patients. *J Clin Invest* 1992; **90**: 1679.
31. Indo Y, Glassberg R, Yokota I, Tanaka K. Molecular characterization of variant alpha-subunit of electron transfer and identification of glycine substitution for valine-157 in the sequence of the precursor producing an unstable mature protein in a patient. *Am J Hum Genet* 1991; **49**: 575.
32. Beard SE, Spector EB, Seltzer WK et al. Mutations in electron transfer flavoprotein:ubiquinone oxidoreductase (ETF:QO) in glutaric acidemia type II (GA2). *Clin Res* 1993; **41**: 271A.
33. Sweetman L, Nyhan WL, Trauner DA et al. Glutaric aciduria type II. *J Pediatr* 1980; **96**: 1020.
34. Nyhan WL, Sakati NO. Glutaric aciduria type 2. In: *Diagnostic Recognition of Genetic Disease*. Philadelphia, PA: Lea and Febiger, 1987: 77.
35. Lehnert W, Wendel U, Lindermaier S, Böhm N. Multiple acyl-CoA dehydrogenation deficiency (glutaric aciduria type II) congenital polycystic kidneys and symmetric warty dysplasia of the cerebral cortex in two brothers. I. Clinical metabolic and biochemical findings. *Eur J Pediatr* 1982; **139**: 56.
36. Böhm N, Uy J, Kiessling M, Lehner W. Multiple acyl-CoA dehydrogenation deficiency (glutaric aciduria type II) congenital polycystic kidneys and symmetric warty dysplasia of the cerebral cortex in two newborn brothers. *Eur J Pediatr* 1982; **139**: 60.
37. Gregersen N, Kolvraa S, Rasmussen K et al. Biochemical studies in a patient with defects in the metabolism of acyl CoA and sarcosine: another possible case of glutaric aciduria type II. *J Inherit Metab Dis* 1980; **3**: 67.
38. Coude FX, Ogier H, Charpentier C et al. Neonatal glutaric aciduria type II: an X-linked recessive inherited disorder. *Hum Genet* 1981; **59**: 63.
39. Goodman SI, Reale M, Berlow S. Glutaric acidemia type II: a form with deleterious intrauterine effects. *J Pediatr* 1983; **102**: 411.
40. Goodman SI, Frerman FE. Glutaric acidemia type II (multiple acyl-CoA dehydrogenation deficiency). *J Inherit Metab Dis* 1984; **7**: 33.
41. Harkin JC, Gill WL, Shapira E. Glutaric acidemia type II: phenotypic findings and ultrastructural studies of brain and kidney. *Arch Pathol Lab Med* 1986; **110**: 399.
42. Mitchell G, Saudubray JM, Gubler MC et al. Congenital anomalies in glutaric aciduria type 2. *J Pediatr* 1984; **104**: 961.
43. Wilson GN, de Chadarevian J-P, Kaplan P et al. Glutaric aciduria type II: review of the phenotype and report of an unusual glomerulopathy. *Am J Med Genet* 1989; **32**: 395.
44. Goodman SI, McCabe ERB, Fennessey PV, Mace JW. Multiple acyl-CoA dehydrogenase deficiency (glutaric aciduria type II) with transient hypersarcosinemia and sarcosinuria: possible inherited deficiency of an electron transfer flavoprotein. *Pediatr Res* 1980; **14**: 12.
45. Goodman SI, Stene DO, McCabe ERB et al. Glutaric acidemia type II. Clinical biochemical and morphologic considerations. *J Pediatr* 1982; **100**: 946.
46. Niederwieser A, Seimann B, Exner U et al. Multiple acyl-CoA dehydrogenation deficiency (MADD) in a boy with nonketotic hypoglycemia hepatomegaly muscle hypotonia and cardiomyopathy. Detection of N-isovalerylglutamic acid and its monoamide. *Helv Paediatr Acta* 1983; **38**: 9.
47. Bennett MJ, Curnock DA, Engel PC et al. Glutaric aciduria type II. Biochemical investigation and treatment of a child diagnosed prenatally. *J Inherit Metab Dis* 1984; **7**: 57.
48. Angle B, Burton BK. Risk of sudden death and acute life-threatening events in patients with glutaric acidemia type II. *Molec Genet Metab* 2008; **93**: 36.
49. Mantagos S, Genel M, Tanaka K. Ethylmalonic-adipic aciduria: *in vivo* and *in vitro* studies indicating deficiency of activities of multiple acyl-CoA dehydrogenase. *J Clin Invest* 1979; **64**: 1580.
50. Dusheiko G, Kew MC, Joffe BI et al. Glutaric aciduria type II: a cause of recurrent hypoglycemia in an adult. *N Engl J Med* 1979; **301**: 1405.
51. Verge ZH, Sherwood WG. Multiple acyl-CoA dehydrogenase deficiency: a neonatal onset case responsive to treatment. *J Inherit Metab Dis* 1985; **8**: 137.
52. Green A, Marshall TG, Bennett MJ et al. Riboflavin-responsive ethylmalonic-adipic aciduria. *J Inherit Metab Dis* 1985; **8**: 67.
53. Gregersen G, Wintzensen H, Kolvraa S et al. C₆-C₁₀ Dicarboxylic aciduria: investigations of a patient with riboflavin responsive multiple acyl-CoA dehydrogenation defects. *Pediatr Res* 1982; **16**: 861.
54. Mongini T, Doriguzzi C, Palmucci L et al. Lipid storage myopathy in multiple acyl-CoA dehydrogenase deficiency: an adult case. *Eur Neurol* 1992; **32**: 170.
55. Cornelio F, DiDonato S, Peluchetti D et al. Fatal cases of lipid storage myopathy with carnitine deficiency. *J Neurol Neurosurg Psychiatry* 1977; **40**: 170.
56. DiDonato S, Frerman FE, Rimondi M et al. Systemic carnitine deficiency due to lack of electron transfer flavoprotein:ubiquinone oxido-reductase. *Neurology* 1986; **36**: 957.

57. Liang WC, Ohkuma A, Hayashi YK *et al.* ETFDH mutations, CoQ10 levels, and respiratory chain activities in patients with riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency. *Neuromuscul Dis* 2009; **19**: 212.
58. Haas R, Nyhan WL. Disorders of organic acids. In: Berg B (ed.). *Neurologic Aspects of Pediatrics*. Boston, MA: Butterworth-Heinemann, 1992: 47.
59. Stöckler S, Radner H, Karpf EF *et al.* Symmetric hypoplasia of the temporal cerebral lobes in an infant with glutaric aciduria type II (multiple acyl-coenzyme A dehydrogenase deficiency). *J Pediatr* 1994; **124**: 601.
60. Jacobs C, Sweetman L, Wadman SK *et al.* Prenatal diagnosis of glutaric aciduria type II by direct chemical analysis of dicarboxylic acids in amniotic fluid. *Eur J Pediatr* 1984; **141**: 153.
61. Chalmers RA, Tracy BM, King GS *et al.* The prenatal diagnosis of glutaric aciduria type II using quantitative GC/MS. *J Inherit Metab Dis* 1985; **8**: 145.
62. Mitchell G, Saudubray JM, Benoit Y *et al.* Antenatal diagnosis of glutaric aciduria type II. *Lancet* 1983; **1**: 1099.
63. Yamaguchi S, Shimizu N, Orii T *et al.* Prenatal diagnosis and neonatal monitoring of a fetus with glutaric aciduria type II due to electron transfer flavoprotein (β -subunit) deficiency. *Pediatr Res* 1991; **30**: 439.
64. Husain M, Stankovich MT, Fox BG. Measurement of the oxidation-reduction potentials for one-electron and two-electron reduction of electron transfer flavoprotein from pig liver. *Biochem J* 1984; **219**: 1043.
65. Ferman FE. Reaction of electron transfer flavoprotein ubiquinone oxidoreductase with the mitochondrial respiratory chain. *Biochim Biophys Acta* 1987; **893**: 161.
66. Yamaguchi S, Orii T, Suzuki Y *et al.* Newly identified forms of electron transfer flavoprotein deficiency in two patients with glutaric aciduria type II. *Pediatr Res* 1991; **29**: 60.
67. Bell RB, Brownell AKW, Roe CR *et al.* Electron transfer flavoprotein: ubiquinone oxidoreductase (ETF:QO) deficiency in adult. *Neurology* 1990; **40**: 1779.
68. Ikeda Y, Keese SM, Tanaka K. Biosynthesis of electron transfer flavoprotein in a cell-free system and in cultured human fibroblasts. Defect in the alpha subunit synthesis is a primary lesion in glutaric aciduria type II. *J Clin Invest* 1986; **78**: 997.
69. Yamaguchi S, Orii T, Maeda K *et al.* A new variant of glutaric aciduria type II deficiency of β -subunit of electron transfer flavoprotein. *J Inherit Metab Dis* 1990; **13**: 783.
70. Loehr J, Ferman FE, Goodman SI. A new form of glutaric acidemia type II (GA2). *Pediatr Res* 1987; **21**: 291A (Abstr.).
71. Dubiel B, Dabrowski C, Wetts R, Tanaka K. Complementation studies of isovaleric academia and glutaric aciduria type II using cultured skin fibroblasts. *J Clin Invest* 1983; **72**: 1543.
72. Rhead WJ, Freneaux E, Sheffield VC *et al.* Glutaric acidemia type II (GAIL): heterogeneity in beta-oxidation flux polypeptide synthesis and cDNA mutations in the alpha-subunit of electron transfer flavoprotein in 8 patients. *Am J Hum Genet* 1992; **25**: A175.
73. Olsen RKJ, Andresen BS, Christensen E *et al.* Clear relationship between ETF/ETFDH genotype and phenotype in patients with multiple acyl-CoA dehydrogenation deficiency. *Hum Mutat* 2003; **22**: 12.
74. Colombo I, Finocchiaro G, Garavaglia B *et al.* Mutations and polymorphisms of the gene encoding the beta-subunit of the electron transfer flavoprotein in three patients with glutaric acidemia type II. *Hum Molec Genet* 1994; **3**: 429.
75. Christensen E, Kolvraa S, Gregersen N. Glutaric aciduria type II: evidence for a defect related to the electron transport flavoprotein or its dehydrogenase. *Pediatr Res* 1984; **18**: 663.
76. Christensen E. Glutaryl CoA dehydrogenase activity determined with intact electron-transport chain: application to glutaric aciduria type II. *J Inherit Metab Dis* 1984; **7**: 103.
77. Moon A, Rhead WJ. Complementation analysis of fatty acid oxidation disorders. *J Clin Invest* 1987; **79**: 59.
78. Gregersen N, Wintzensen H, Kolvraa S *et al.* C₆-C₁-dicarboxylic aciduria: investigations of a patient with riboflavin-responsive multiple acyl-CoA dehydrogenation defects. *Pediatr Res* 1982; **16**: 861.
79. Chalmers RA, Roe CR, Stacey TE, Hoppel CL. Urinary excretion of L-carnitine and acylcarnitines by patients with disorders of organic acid metabolism: evidence for secondary insufficiency of L-carnitine. *Pediatr Res* 1984; **18**: 1325.
80. Mandel H, Africk D, Blitzer M, Shapira E. The importance of recognizing secondary carnitine deficiency in organic acidemias: case report in glutaric acidemia type II. *J Inherit Metab Dis* 1988; **11**: 397.
81. Vreken P, van Lint AEM, Bootsma AH *et al.* Rapid diagnosis of organic acidemias and fatty acid oxidation defects by quantitative electrospray tandem-MS acyl-carnitine analysis in plasma. In: Quant P, Eaton S (eds). *Current Views of Fatty Acid Oxidation and Ketogenesis: From Organelles to Point Mutations*. New York: Plenum Publishers, Kluwer Academic, 1999: 327.
82. Poplawski NK, Ranieri E, Harrison JR, Fletcher JM. Multiple acyl-CoA dehydrogenase deficiency: diagnosis by acyl-carnitine analysis of a 12-year-old newborn screening card. *J Pediatr* 1999; **134**: 764.
83. De Visser M, Scholte HR, Schutgens RBH. Riboflavin-response lipid-storage myopathy and glutaric aciduria type II of early adult onset. *Neurology* 1986; **36**: 367.
84. Uziel G, Garavaglia B, Ciceri E *et al.* Riboflavin-responsive glutaric aciduria type II presenting as a leukodystrophy. *Pediatr Neurol* 1995; **13**: 333.
85. Rinaldo P, Welch RD, Previs SF *et al.* Ethylmalonic/adipic aciduria: effects of oral medium-chain triglycerides carnitine and glycerol on urinary excretion of organic acids acylcarnitines and acylglycines. *Pediatr Res* 1991; **30**: 216.
86. Bosch AM, Nico GGMA, Ijlst L *et al.* Brown-Vialetto-Van Laere and Fazio Londe syndrome is associated with a riboflavin transporter defect mimicking mild MADD: a new inborn error of metabolism with potential treatment. *J Inherit Metab Dis* 2011; **34**: 159.
87. Green P, Wiseman M, Crow YJ *et al.* Brown-Vialetto-Van Laere syndrome, a pontobulbar palsy with deafness, is caused by mutations in c20orf54. *Am J Hum Genet* 2010; **86**: 485.

3-Hydroxy-3-methylglutarylCoA lyase deficiency

Introduction	325	Treatment	331
Clinical abnormalities	326	References	331
Genetics and pathogenesis	329		

MAJOR PHENOTYPIC EXPRESSION

Hypoketotic hypoglycemia, metabolic acidosis, hyperammonemia; hepatomegaly; a characteristic organic aciduria: 3-hydroxy-3-methylglutaric, 3-methylglutaconic, 3-methylglutaric, and 3-hydroxyisovaleric acids; and deficiency of 3-hydroxy-3-methylglutarylCoA lyase.

INTRODUCTION

3-Hydroxy-3-methylglutaric aciduria is a disorder of leucine metabolism (Figure 45.1) that leads to life-threatening illness early in life. Once diagnosed, management, particularly the avoidance of fasting, can be very rewarding. The first patient was reported in 1976 by Faull and colleagues [1]. This infant was well until seven months of age when he developed diarrhea and vomiting, and within 24 hours he had lethargy, pallor, dehydration,

cyanosis, and apnea, requiring resuscitation. At four years and seven months [2], development was satisfactory. The disorder has been encountered frequently among Arab families [3].

Hydroxymethylglutaryl (HMG)-CoA lyase deficiency may be considered an organic acidemia. It is at the same time a classic disorder of fatty acid oxidation. HMG-CoA lyase is the last step in the formation of acetoacetate (Figure 45.2) and its product, 3-hydroxybutyrate. The products of the cleavage of HMG-CoA are acetoacetate and acetylCoA.

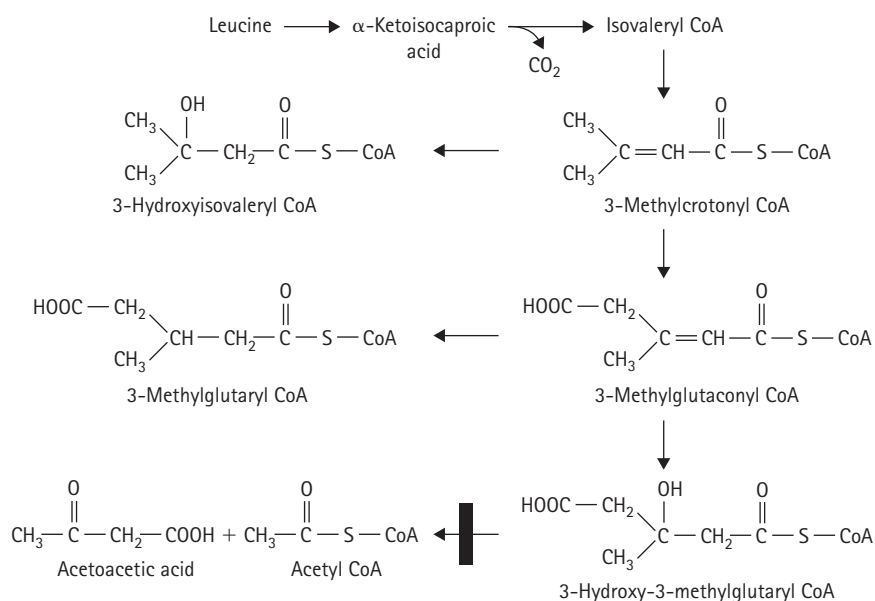


Figure 45.1 The pathway of the catabolism of leucine and 3-hydroxy-3-methylglutaryl (HMG) CoA lyase, the site of the defect in HMG aciduria.

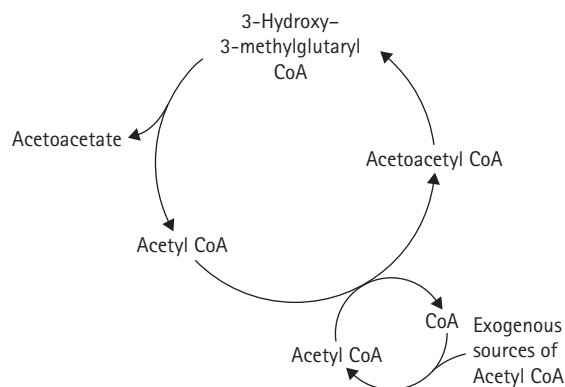


Figure 45.2 Ketogenesis. 3-Hydroxy-3-methylglutaryl (HMG) CoA and its lyase play a critical role.

Of course, HMG-CoA is also a key intermediate in the synthesis of cholesterol ([Chapter 85](#)). Its reduction to mevalonic acid represents a feedback control point in this pathway.

CLINICAL ABNORMALITIES

The classic presentation is with a Reye syndrome-like episode in late infancy (from six months to two years), usually following an intercurrent infection which leads to vomiting or failure to eat [1–8], some present in the neonatal period, but the majority between three and 11 months. The disease has also been reported to be manifested clinically in adults. A 36-year-old woman with seizures and severe leukoencephalopathy, and a 29-year-old adult with no prior history of the disease have been reported [9, 10].

Persistent vomiting may be an early symptom. There is rapid progression from lethargy and hypotonia to coma. Pallor and dehydration are commonly present. There may be seizures, including myoclonus. Hypothermia has been reported [11]. Apnea is followed by death unless the patient is artificially ventilated. Clinical chemical evaluation reveals hypoglycemia, metabolic acidosis, and, in some, hyperammonemia. For this reason, a number of patients have initially been diagnosed as Reye syndrome [6, 7]. Presentation with life-threatening acidosis is common [4, 8, 11]. Infants may present in the first days of life with seizures, lethargy, or tachypnea. This may follow the first feeding or may precede it, an index that birth itself maybe a catabolic experience. Lactic acidemia is prominent. The initial episode may be fatal [8, 11].

Recurrent episodes of acute illness have been observed particularly in those who presented in the neonatal period [8]. The patient is always at risk of acute illness, if infection or other problem leads to fasting. Some families have learned to intervene sufficiently, promptly, and effectively that episodes have been prevented or aborted.



Figure 45.3 An eight-year-old girl who was diagnosed as having 3-hydroxy-3-methylglutaryl (HMG) Co lyase deficiency in the first month of life when she presented with severe hypoglycemia (blood sugar was 0.5 mmol/L), negative urine for ketones, and convulsions. She required anticonvulsant medications.

Hepatomegaly is a regular occurrence [12], and there may be elevation of levels of transaminases in the blood. However, hepatomegaly may be absent especially in the neonatal presentation. It has been absent in a nine-month old, who had elevated transaminases [13]. Histologic examination of the liver reveals infiltration of fat.

Brain injury may result from hypoglycemia, shock, or both. Some patients have had a persistent seizure disorder and abnormalities of the electroencephalogram (EEG) ([Figure 45.3](#)) [6, 8]. Microcephaly has been observed in several patients [14, 15]. One patient had impaired mental development was macrocephalic [16]. Hemiparesis has been reported [17], as well as decerebrate tetraparesis [8]. Impaired mental development may be severe [8, 18]. On the other hand, most patients are developmentally normal. There are no dysmorphic features ([Figures 45.4, 45.5, 45.6, 45.7, 45.8, and 45.9](#)).

One patient ([Figure 45.6](#)) presented at five years of age with pernicious vomiting and abdominal tenderness, and was found to have acute pancreatitis [19]. She had recurrent episodes of hypoglycemia and acidosis. Pancreatitis has been described increasingly in patients with Reye syndrome [20] and in inborn errors of metabolism [21], suggesting further commonalities in pathogenesis of other metabolic disorders thought to be Reye syndrome.

Magnetic resonance imaging (MRI) of the brain reveals increased T_2 signal indicating hypodensity of white matter ([Figures 45.10 and 45.11](#)) [8, 13, 16]. In patients in whom brain damage has occurred, the picture may be that of cerebral atrophy ([Figure 45.12](#)) [8, 18].



Figure 45.4 HHHA: A one-month-old infant with 3-hydroxy-3-methylglutaryl (HMG) CoA lyase deficiency. She developed lactic acidosis and coma at 5 days of age and was found to have 668 mmol/creatinine of HMG in the urine. Activity of HMG CoA lyase in fibroblasts was 0.9 percent of control. The tonic neck reflex is normal at her age. Examination was unremarkable.



Figure 45.5 HHHA: There were no dysmorphic features. Magnetic resonance imaging showed some evidence of cerebral atrophy.



Figure 45.6 A girl with 3-hydroxy-3-methylglutaric aciduria. She was admitted to hospital at five years of age with acute pancreatitis. The activity of 3-hydroxy-3-methylglutaryl CoA lyase in lymphocytes and fibroblasts was 2 percent of normal. (The illustration was kindly provided by Dr William Wilson of the University of Virginia.)



Figure 45.7 N: A three-year-old patient with 3-hydroxy-3-methylglutaryl (HMG) CoA lyase deficiency. She developed severe neonatal lactic acidosis, hypoglycemia and coma. Despite noncompliance with dietary treatment and multiple acidotic attacks, she was developing normally. By follow up at seven years of age, she was doing better than average in school, although attacks were continuing.

The hypoglycemic acute episode is striking, often extreme and with a notable absence of ketosis. Initial concentrations of sugar in the blood have ranged from 0.2 to 1.8 mmol/L; many were below 0.4 mmol/L (8 mg/dL) and one patient died in a second episode of hypoglycemia



Figure 45.8 S: A three-year-old girl with 3-hydroxy-3-methylglutaryl (HMG) CoA lyase deficiency. She had severe lactic acidotic episodes in the neonatal period and at five and 14 months, in the latter of which she developed shock and convulsions along with an unmeasurable blood glucose. On follow up at seven years of age, she had no attacks for three years and was developing normally.

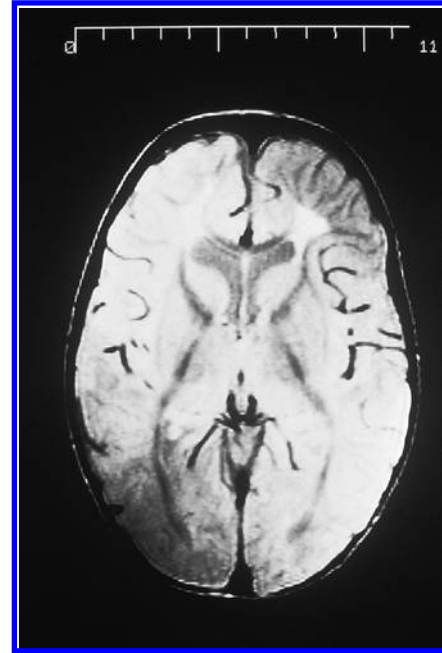


Figure 45.10 Magnetic resonance imaging of the brain of the patient in Figures 45.4 and 45.5 at 2.5 years reveals increased signal intensity in the frontal white matter. (Reprinted with permission from the *Journal of Inherited Metabolic Disease* [8], with kind permission of Springer Science & Business Media.)

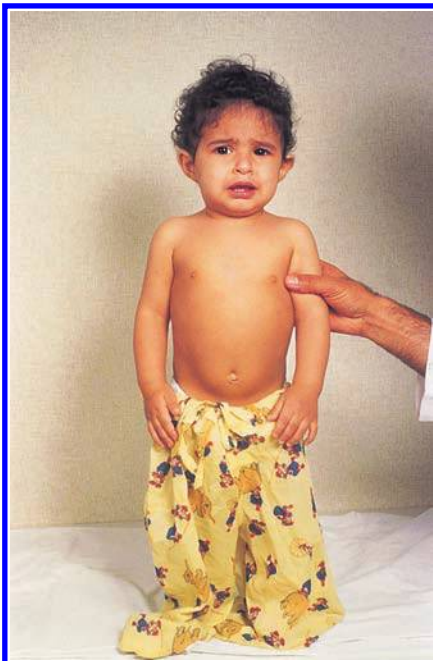


Figure 45.9 A 21-month-old boy with 3-hydroxy-3-methylglutaryl (HMG) CoA lyase deficiency. His first acidotic episode was at six months of age following herpangina and refusal to eat. At six years, he had not had an attack in three years and was doing very well in school.

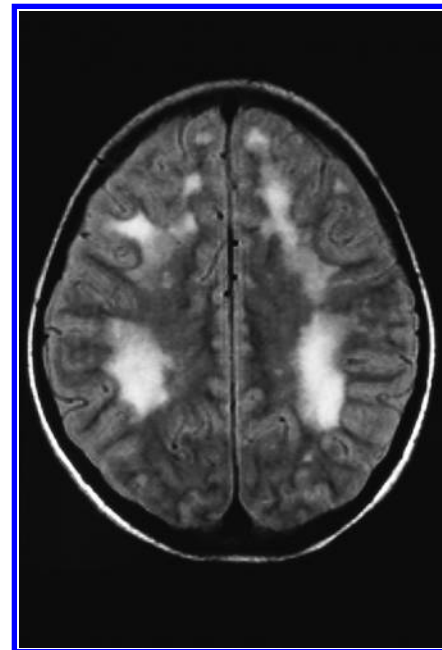


Figure 45.11 Magnetic resonance image of the brain of a nine-year-old with 3-hydroxy-3-methylglutaryl (HMG) CoA lyase deficiency. There was extensive increase in signal in the subcortical white matter consistent with dysmyelination. (Kindly provided by Dr Robert Schwartz of Brown University School of Medicine.)

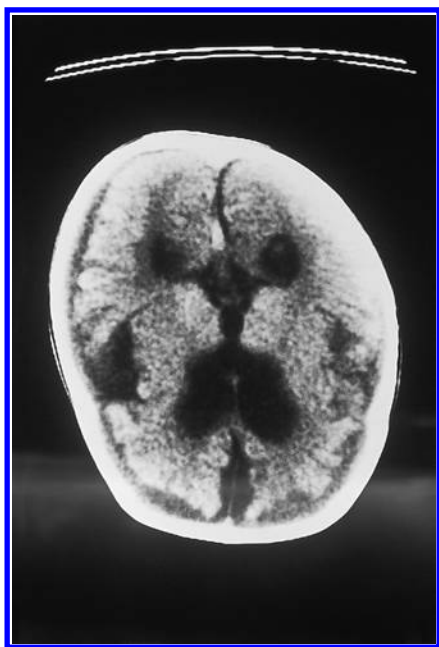


Figure 45.12 The sister of the patient in Figure 45.8. She was diagnosed late. The computed tomography (CT) scan at ten months shows considerable evidence of cerebral atrophy. She was severely impaired neurologically and died at 19 months.

in which the concentration recorded was less than 0.1 mmol/L. At this time, the concentration of insulin was less than 1 mU/L. The episode had followed a change in diet in which the amounts of leucine ingested were increased. The infant developed cyanosis, vomiting, and hypotonia. In three patients, the values for the blood pH were recorded as 7.24, 7.11, and 7.29 [6, 7, 11]. During the acute crisis, a pH below 7.0 is not unusual especially in the neonatal onset patients [4, 8]. The initial plasma concentrations of bicarbonate were below 16 mEq/L in five patients [4]. Lactic acidosis has been documented with levels as high as 10 and 20 mmol/L [4]. Persistent infantile hypoglycemia in the presence of metabolic acidosis is an indication for organic acid analysis.

Hyperammonemia has been observed in about 50 percent of patients [4]. In three patients, concentrations ranged from 388 to 1370 $\mu\text{mol/L}$ [4] and in one patient the plasma concentration of ammonia was greater than 2000 $\mu\text{mol/L}$ [3]. Abnormal liver function tests included alanine and aspartate aminotransferase [1, 6]; bilirubin, gamma-glutamyl transpeptidase (GGT) [11], and prolonged prothrombin time [6, 7], all of which lead to confusions with a diagnosis of Reye syndrome. In fact, the provisional diagnosis was Reye syndrome in the first four patients reported. With successful treatment, the abnormalities in liver function disappear. The prognosis is guarded. Death has been observed in at least five patients [8, 11, 17, 18, 22].

None of these patients had ketonuria at times of acute illness, and in some low levels of acetoacetate and

3-hydroxybutyrate have been documented in the blood [7, 17, 18, 23]. Levels of acetoacetate and 3-hydroxybutyrate in the urine are disproportionately low. This serves to distinguish these patients from those with other organic acidurias, such as propionic acidemia (Chapter 2) or methylmalonicacidemia (Chapter 3). This is consistent with the site of the defect (Figure 45.2) in which 3-hydroxy-3-methylglutaric acid cannot be converted to acetoacetic acid and acetylCoA. Ketone bodies decrease proteolysis in muscle and conserve muscle protein during starvation [24]; so impairment of ketogenesis could be relevant to the hyperammonemia.

GENETICS AND PATHOGENESIS

HMG-CoA lyase deficiency is transmitted as an autosomal recessive trait. Consanguinity has been observed in a number of families [6, 8, 11]. The molecular defect is in the enzyme HMG-CoA lyase (Figure 45.1). Defective activity of the enzyme has been demonstrated in cultured fibroblasts [25, 26], leukocytes [27], and liver [11] of affected patients. Activity in ten patients was reported to be undetectable; in 16 others, it ranged from 0.7 to 13.7 percent of normal [4]. A variety of methods is available for enzyme analysis [28], including direct detection by high performance liquid chromatography (HPLC) of the product of the reaction [26]. In addition, the defect can be identified by measuring the incorporation of ^{14}C -isovaleric acid into trichloroacetic acid precipitable macromolecules [29] or by monitoring metabolism of ^{14}C -leucine [30].

Mutation at Arg41 was found in a patient with severe deficiency of the enzyme [31] which is consistent with a role for interaction of arg41 with the acylCoA carbonyl, promoting product enolization.

The crystal structure of HMG CoA lyase has been published [31]; it has confirmed a barrel structure predicted by molecular modeling [32–34] in which there is a carboxyl end cavity formed by eight β -strands of the barrel, which have one molecule of 3-hydroxyglutaric acid and Mg^{2+} . Substrate binding involves asparagine 311 and lysine 313 and the establishment of polar contacts with phosphate and ribose groups of adenosine [32]. A G-loop structure would facilitate a disulfide bond between cysteine 266 and cysteine 323 [31].

The activity of the HMG CoA lyase in leukocyte or fibroblasts in the parents of patients is intermediate between those of the patient and controls [4, 26, 27]. However, in some families, obligate heterozygotes have had normal values. Study of expression at the levels of mRNA, protein, and enzyme activity in various tissues revealed greatest activity in liver, followed unexpectedly by pancreas. In heart and adult brain, activity was not detected. These findings are consistent with the occurrence of pancreatitis in this disease. The striatum has been viewed as particularly vulnerable to oxidative damage by 3-hydroxy-3methylglutarate, which is a strong pro-oxidant [35].

Prenatal diagnosis has been accomplished by the analysis of metabolites in maternal urine at 23 weeks of gestation [36]. The enzyme is active in amniocytes [37]. Prenatal diagnosis should be possible by direct measurement of hydroxymethylglutamate by stable isotope dilution gas chromatography-mass spectrometry (GCMS) of the amniotic fluid.

The gene for HMG CoA lyase has been cloned [38]. The sequence predicts a 27 residue mitochondrial leader and a 31.6-kDa mature protein. A number of mutations have provided interesting information on the nature of the enzyme. Five mutations in the highly conserved R41 and D42 codons were found in 23 percent of the mutant alleles in 41 probands. They were R41Q, R41X, D42H, D42G, and D42E [39]. R41Q is common in Saudi Arabia where six of nine probands were homozygous. This mutation has also been found in Turkish and Italian patients. Among major alterations in the gene were two large deletions [40] and three frame shift/premature terminations [38,39]. Two stop codon mutations and a 2-bp deletion led to alternatively spliced mRNA [41–43]. Among 93 patients reported [33], 30 variant alleles were found (28 mutations and two SNPs). Particular frequency was noted in Saudi Arabia and the Iberian peninsula where two mutations (122G>A and 109G>A) have been identified in 87 and 94 percent. Some clustering was observed in exon 2 [33]. In Brazil, two mutations were predominant in ethnic Portuguese/Spanish (E37X and V168fs (-2)) [44]. A single (E37X (c.109G>T)) was found in 84 percent of alleles from northern Portugal [45]. In Taiwanese patients [46] c.494G>T, p.Arg165Gln and two splice site mutations (IVS3+1G>A and IVS6-1G>A) leading to skipping of exon 3 were the predominant

mutations in this population. Despite these elegant studies, clear relationship of genotype to phenotype has not emerged. It is possible that this reflects the fact that clinical manifestations more likely result from hypoglycemia and its consequences than from severity of reduction in enzyme activity.

The pattern of organic aciduria in this disorder is characteristic (Figure 45.13) [47–50]. 3-Hydroxy-3-methylglutaric acid is excreted in appreciable quantities in the urine. In acute crisis, levels may reach 10,000–20,000 mmol/mol creatinine and, between crises, may be 200–4000. Normal individuals excrete in urine less than 100 mmol/mol creatinine [49]; levels are somewhat higher in young infants. Organic acid analysis by GCMS usually involves trimethylsilyl derivatives, but in the case of 3-hydroxy-3-methylglutaric acid, appreciable quantities of the di-derivative are formed as well as the tri-derivative, as both must be included for quantification [51]. Large amounts of 3-methylglutaconic acid are also found in the urine. These compounds represent successive steps in the catabolism of leucine (Figure 45.1) in which isovalerylCoA is converted to 3-methylcrotonylCoA which is then converted to 3-methylglutaconylCoA. The addition of H₂O across the double bond in this compound yields 3-hydroxy-3-methylglutarylCoA, which is ultimately cleaved to form acetoacetic acid and acetylCoA. 3-Methylglutaric acid is also found; this would result from reduction of 3-methylglutaconic acid. The reaction could be catalyzed by the enzyme that catalyzes the reverse dehydrogenation of 3-methylcrotonylCoA to isovalerylCoA. In addition, 3-hydroxyisovaleric acid is also found in the urine [50]. This compound would arise

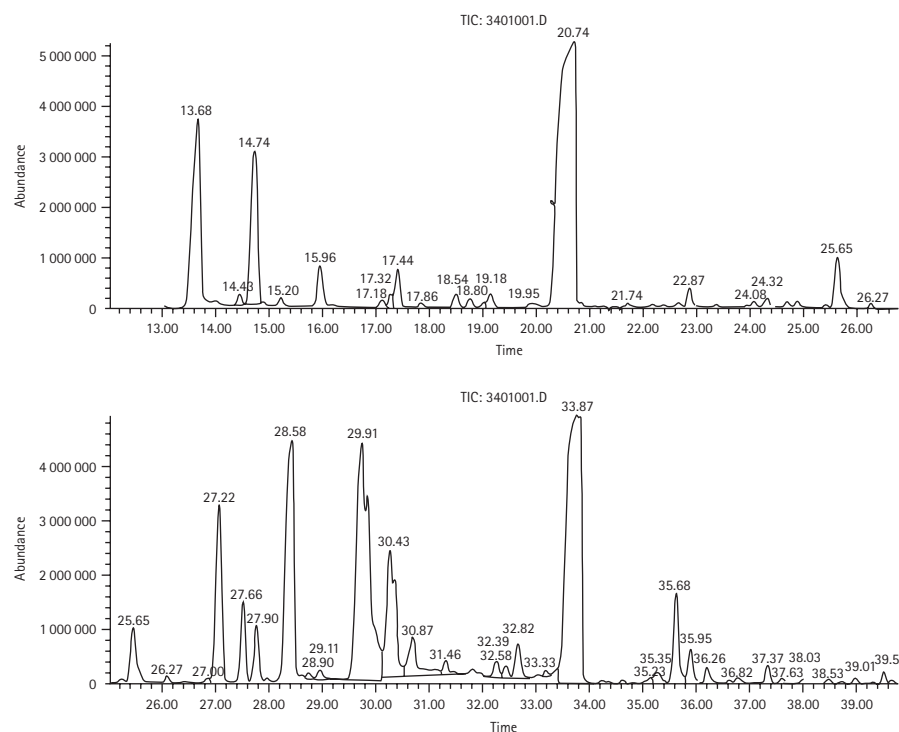


Figure 45.13 Organic acid analysis of the urine of a patient with 3-hydroxy-3-methylglutaryl (HMG) CoA lyase deficiency. The important compounds were: 3-hydroxyisovaleric acid at 20.74, 3-methylglutaric acid at 27.90, 3-methylglutaconic acid at 28.58 and 29.91, and 3-hydroxy-3-methylglutaric acid at 33.87.

from the hydration of 3-methylcrotonylCoA. In the acute episode, a large elevation of 3-hydroxyisovaleric acid may be found, along with 3-methylcrotonylglycine [19, 50–52]. Glutaric acid and adipic may also be elevated in the urine in the acute crisis [53]. Lactic acid levels may be elevated at these times [54], along with hyperammonemia. Levels of acetoacetate and 3-hydroxybutyrate are disproportionately low in the urine, as they are in the blood.

3-Methylcrotonic (3-methyl-2-butenic) acid may be found in the urine, along with its isomer, 3-methyl-3-butenic acid [47], but these are artifacts of the GCMS analysis [53, 55]. The abnormal metabolites in this disease may also be detected by nuclear magnetic resonance (NMR) spectroscopy [56] permitting rapid diagnosis. Rapid diagnosis is now more likely to be made by tandem mass spectrometry (MS/MS). 3-Methylglutaryl-carnitine has been found in the plasma and urine [57], and 3-hydroxy-isovaleryl-carnitine has been found in plasma (see Table 1.2) [58]. This permits the incorporation of this disease into programs of neonatal screening.

The excretion of acylcarnitine esters is elevated in this condition and there may be a secondary deficiency of free carnitine [59].

TREATMENT

Management of the patient should be considered under two headings, long-term management and treatment of the acute crisis. The latter is an emergency and care must be devoted first to measures of general support, such as assisted ventilation and repair of deficits of fluid and electrolytes, and elevation of the blood concentration of glucose. In an infant with or without hyperammonemia, exchange transfusion, or peritoneal dialysis may be necessary. Sodium benzoate or phenylacetate and L-arginine are useful in management of the hyperammonemia [60]. Hypoglycemia and acidosis usually respond readily to the parenteral administration of 20 percent glucose with intravenous insulin to keep blood sugar between 6 and 8 mmol/L, fluid, and electrolytes. Parents should be instructed to bring the patient in early, whenever the oral route is compromised by vomiting or anorexia.

Long-term management rests largely on the avoidance of hypoglycemia and the avoidance of long fasting, especially during intercurrent illness. The importance of ketogenesis in glucose homeostasis was illustrated by the prevention of hypoglycemia with fasting in a patient with HMG CoA lyase deficiency by the infusion of 3-hydroxybutyrate [23]. Frequent feedings are advisable in infancy, with even sleeping through the night permitted only after it is documented that this does not lead to hypoglycemia. A high carbohydrate diet with added cornstarch is advisable and supplementation with glucose polymers is convenient, especially during intercurrent illness. Cornstarch is useful at bedtime.

Restriction of the intake of protein has been employed,

and it appears prudent to restrict the amounts of leucine ingested. Restriction of the intake of fats may reduce the levels of metabolites in the urine [12]. Carnitine supplementation has proven to be a useful adjunct to therapy [61].

REFERENCES

1. Faull K, Bolton P, Halpern B *et al.* Patient with defect in leucine metabolism. *N Engl J Med* 1976; **294**: 1013.
2. Shilkin R, Wilson G, Owles E. 3-Hydroxy-3-methylglutaryl coenzyme A lyase deficiency. Follow-up of first described case. *Acta Paediatr Scand* 1981; **70**: 265.
3. Ozand PT, DeVol EB, Gascon GG. Neurometabolic diseases at a national referral center: five years experience at the King Faisal Specialist Hospital and Research Centre. *J Child Neurol* 1992; **7**: S4.
4. Gibson KM, Breuer J, Nyhan WL. 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency: review of 18 reported patients. *Eur J Pediatr* 1988; **148**: 180.
5. Gibson KM, Lee CL, Kamail V *et al.* 3-Hydroxy-3-methylglutaryl coenzyme A lyase deficiency as detected by radiochemical assay in cell extracts by thin layer chromatography and identification of two new cases. *Clin Chem* 1990; **36**: 297.
6. Leonard JV, Seakins JWT, Griffin NK. β -Hydroxy- β -methylglutaric aciduria presenting as Reye syndrome. *Lancet* 1979; **1**: 680.
7. Robinson BH, Oei J, Sherwood WG *et al.* Hydroxymethylglutaryl CoA lyase deficiency: features resembling Reye syndrome. *Neurology* 1980; **30**: 714.
8. Ozand PT, Al Aqeel A, Gascon GG *et al.* 3-Hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA) lyase deficiency in Saudi Arabia. *J Inherit Metab Dis* 1991; **14**: 174.
9. Reimão S, Morgado C, Almeida IT *et al.* 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency: initial presentation in a young adult. *J Inherit Metab Dis* 2009, Feb 24 [Epub ahead of print].
10. Bischof F, Nägele T, Wanders RJ *et al.* 3-hydroxy-3-methylglutaryl-CoA lyase deficiency in an adult with leukoencephalopathy. *Ann Neurol* 2004; **56**: 727.
11. Schutgens RB, Heymans H, Ketel A *et al.* Lethal hypoglycemia in a child with a deficiency of 3-hydroxy-3-methylglutaryl-coenzyme A lyase. *J Pediatr* 1979; **94**: 89.
12. Norman EJ, Denton MD, Berry HK. Gas chromatographic/mass spectrometric detection of 3-hydroxy-3-methylglutaryl-CoA lyase deficiency in double first cousins. *Clin Chem* 1982; **28**: 137.
13. Gibson KM, Breuer J, Kaiser K *et al.* 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency: report of five new patients. *J Inherit Metab Dis* 1988; **11**: 76.
14. Lisson G, Leupold B, Bechinger D, Wallesch C. CT findings in a case of deficiency of 3-hydroxy-3-methylglutaryl-CoA lyase. *Neuroradiology* 1981; **22**: 99.
15. Stacey TE, de Sousa C, Tracey BM *et al.* Dizygotic twins with 3-hydroxy-3-methylglutaric aciduria: unusual presentation family studies and dietary management. *Eur J Pediatr* 1985; **144**: 177.

16. Leupold D, Bojash M, Jakobs C. 3-Hydroxy-3-methylglutaryl-CoA lyase deficiency in an infant with macrocephaly and mild metabolic acidosis. *Eur J Pediatr* 1982; **138**: 73.
17. Zoghbi HY, Spence JE, Beaudet AL *et al*. Atypical presentation and neuropathological studies in 3-hydroxy-3-methylglutaryl-CoA lyase deficiency. *Ann Neurol* 1986; **20**: 367.
18. Walter JH, Clayton PT, Leonard JV. 3-Hydroxy-3-methylglutaryl-CoA lyase deficiency. *J Inherit Metab Dis* 1986; **9**: 287.
19. Wilson WG, Cass MB, Sovik O *et al*. 3-Hydroxy-3-methylglutaryl-CoA lyase deficiency in a child with acute pancreatitis and recurrent hypoglycemia. *Eur J Pediatr* 1984; **142**: 781.
20. Morens DM, Hammar SL, Heicher DA. Idiopathic acute pancreatitis in children. Association with a clinical picture resembling Reye syndrome. *Am J Dis Child* 1974; **128**: 401.
21. Kahler SG, Sherwood WG, Woolf D *et al*. Pancreatitis in patients with organic acidemias. *J Pediatr* 1994; **124**: 239.
22. Divry P, Rolland MO, Teyssier J *et al*. 3-Hydroxy-3-methylglutaric aciduria combined with 3-methylglutaconic aciduria. A new case. *J Inherit Metab Dis* 1981; **4**: 173.
23. Francois B, Bachmann C, Schutgens RBH. Glucose metabolism in a child with 3-hydroxy-3-methyl glutaryl-coenzyme A lyase deficiency. *J Inherit Metab Dis* 1981; **4**: 163.
24. Felig P, Sherwin R, Palailogos G. Ketone utilization and ketone-amino acid interactions in starvation and diabetes. In: Soeling HD, Seufert CD (eds). *Biochemical and Clinical Aspects of Ketone Body Metabolism*. Stuttgart: Thieme, 1978: 166.
25. Wysocki SJ, Hahnel R. 3-Hydroxy-3-methylglutaric aciduria: deficiency of 3-hydroxy-3-methylglutaryl coenzyme A lyase. *Clin Chim Acta* 1976; **71**: 349.
26. Gibson KM, Sweetman L, Nyhan WL *et al*. 3-Hydroxy-3-methylglutaric aciduria a new assay of 3-hydroxy-3-methylglutaryl-CoA lyase using high performance liquid chromatography. *Clin Chim Acta* 1982; **126**: 171.
27. Wysocki SJ, Hahnel R. 3-Hydroxy-3-methylglutaric aciduria 3-hydroxy-3-methylglutaryl-coenzyme A lyase levels in leukocytes. *Clin Chim Acta* 1976; **73**: 373.
28. Gibson KM. Assay of 3-hydroxy-3-methyl-glutaryl-coenzyme A lyase. *Meth Enzymol Branched Chain Amino Acids* 1988; **166**: 219.
29. Sovik O, Sweetman L, Gibson KM, Nyhan WL. Genetic complementation analysis of 3-hydroxy-3-methylglutaryl-coenzyme A lyase deficiency in cultured fibroblasts. *Am J Hum Genet* 1984; **26**: 791.
30. Yoshida I, Sovik O, Sweetman L, Nyhan WL. Metabolism of leucine in fibroblasts from patients with deficiencies in each of the major catabolic enzymes: branched-chain ketoacid dehydrogenase 3-methylcrotonyl-CoA carboxylase 3-methylglutaconyl-CoA hydratase and 3-hydroxy-3-methylglutaryl-CoA lyase. *J Neurogenet* 1985; **2**: 413.
31. Fu Z, Runquist JA, Montgomery C *et al*. Functional insights into human HMG-CoA lyase from structures of Acyl-CoA-containing ternary complexes. *J Biol Chem* 2010; **8**: 285.
32. Carrasco P, Menao S, López-Viñas E *et al*. C-terminal end and aminoacid Lys48 in HMG-CoA lyase are involved in substrate binding and enzyme activity. *Mol Genet Metab* 2007; **91**: 120.
33. Pié J, López-Viñas E, Puisac B *et al*. Molecular genetics of HMG-CoA lyase deficiency. *Mol Genet Metab* 2007; **92**: 198.
34. Puisac B, Arnedo M, Casale CH *et al*. Differential HMG-CoA lyase expression in human tissues provides clues about 3-hydroxy-3-methylglutaric aciduria. *J Inherit Metab Dis* 2010; **33**: 405.
35. Leipnitz G, Seminotti B, Fernandes CG *et al*. Striatum is more vulnerable to oxidative damage induced by the metabolites accumulating in 3-hydroxy-3-methylglutaryl-CoA lyase deficiency as compared to liver. *Int J Dev Neurosci* 2009; **27**: 351.
36. Duran M, Schutgens RBH, Ketel A *et al*. 3-Hydroxy-3-methylglutaryl coenzyme A lyase deficiency: postnatal management following prenatal diagnosis by analysis of maternal urine. *J Pediatr* 1979; **95**: 1004.
37. Hahnel R, Wysocki SJ. Potential prenatal diagnosis of 3-hydroxy-3-methylglutaryl-Coenzyme A lyase deficiency. *Clin Chim Acta* 1981; **111**: 287.
38. Mitchell GA, Robert MF, Hruz PW. 3-Hydroxy-3-methylglutaryl coenzyme A lyase (HL). *J Biol Chem* 1993; **268**: 4376.
39. Mitchell GA, Ozand PT, Robert M-F *et al*. HMG CoA lyase deficiency: Identification of five causal point mutations in codons 41 and 42 including a frequent Saudi Arabian mutation R41Q. *Am J Hum Genet* 1997; **62**: 295.
40. Wang SP, Robert M-F, Gibson KM *et al*. 3-Hydroxy-3-methylglutaryl-CoA lysase (HL): mouse and human HL gene (HMGCL) cloning and detection of large gene deletions in two unrelated HL-deficient patients. *Genomics* 1996; **33**: 99.
41. Buesa C, Pie J, Barcelo A *et al*. Aberrantly spliced mRNAs of the 3-hydroxy-3-methylglutaryl coenzyme A lyase (HL) gene with a donor splice-site point mutation produce hereditary HL deficiency. *J Lipid Res* 1996; **37**: 2420.
42. Pie J, Casals N, Casale CH *et al*. A nonsense mutation in the 3-hydroxy-3-methylglutaric aciduria. *Biochem J* 1997; **323**(Pt 2): 329.
43. Casals N, Pie J, Casale CH *et al*. A two-base deletion in exon 6 of the 3-hydroxy-3-methylglutaryl coenzyme A lyase (HL) gene producing the skipping of exons 5 and 6 determines 3-hydroxy-3-methylglutaric aciduria. *J Lipid Res* 1997; **38**: 2303.
44. Vargas CR, Sitta A, Schmitt G *et al*. Incidence of 3-hydroxy-3-methylglutaryl-coenzyme A lyase (HL) deficiency in Brazil, South America. *J Inherit Metab Dis* 2007; **17**. DOI: 10.1007/s10545-007-0756-y.
45. Cardoso ML, Rodrigues MR, Leão E *et al*. The E37X is a common HMGCL mutation in Portuguese patients with 3-hydroxy-3-methylglutaric CoA lyase deficiency. *Mol Genet Metab* 2004; **82**: 334.
46. Lin WD, Wang CH, Lai CC *et al*. Molecular analysis of Taiwanese patients with 3-hydroxy-3-methylglutaryl CoA lyase deficiency. *Clin Chim Acta* 2009; **401**: 33.
47. Wysocki SJ, Wilkinson SP, Hahnel R *et al*. 3-hydroxy-3-methylglutaric aciduria combined with 3-methylglutaconic aciduria. *Clin Chim Acta* 1976; **70**: 399.
48. Tracey BM, Stacey TE, Chalmers RA. Urinary and plasma organic acids in dizygotic twin siblings with 3-hydroxy-3-methylglutaric aciduria studied by gas chromatography

- and mass spectrometry using fused silica capillary columns. *J Inherit Metab Dis* 1983; **6**: 125.
49. Lippe G, Galsigna L, Rancesconi M *et al*. Age-dependent excretion of 3-hydroxy-3-methylglutaric acid (HMG) and ketone bodies in the urine of full-term and pre-term newborns. *Clin Chim Acta* 1982; **126**: 291.
50. Faull KF, Bolton PD, Halpern B *et al*. The urinary organic acid profile associated with 3-hydroxy-3-methylglutaric aciduria. *Clin Chim Acta* 1976; **73**: 558.
51. Mills GA, Hill MAW, Buchanan R *et al*. 3-Hydroxy-3-methylglutaric aciduria: a possible pitfall in diagnosis. *Clin Chim Acta* 1991; **204**: 131.
52. Wysocki SJ, Hahnel R. 3-Methylcrotonylglycine excretion in 3-hydroxy-3-methylglutaric aciduria. *Clin Chim Acta* 1978; **86**: 101.
53. Duran M, Ketting D, Wadman SK *et al*. Organic acid excretion in a patient with 3-hydroxy-3-methylglutaryl-CoA lyase deficiency. Facts and artifacts. *Clin Chim Acta* 1978; **90**: 187.
54. Green CL, Cann HM, Robinson BH *et al*. 3-Hydroxy-3-methylglutaric aciduria. *J Neurogenet* 1984; **1**: 165.
55. Jakobs C, Bojasch M, Duran M *et al*. An artefact in the urinary metabolic pattern of patients with 3-hydroxy-3-methylglutaryl-CoA lyase deficiency. *Clin Chim Acta* 1980; **106**: 85.
56. Iles RA, Jago JR, Williams SR, Chalmers RA. 3-Hydroxy-3-methylglutaryl-CoA lyase deficiency studied using 2-dimensional proton nuclear magnetic resonance spectroscopy. *FEBS Lett* 1986; **203**: 49.
57. Roe CR, Millington DS, Maltby DA. Identification of 3-methylglutaryl carnitine: a new diagnostic metabolite of 3-hydroxy-3-methylglutaryl-coenzyme A lyase deficiency. *J Biol Chem* 1986; **77**: 1391.
58. van Hove JLK, Rutledge SL, Nada MA *et al*. 3-Hydroxyisovaleryl carnitine in 3-methylcrotonyl-CoA carboxylase deficiency. *J Inherit Metab Dis* 1995; **18**: 592.
59. Chalmers RA, Roe CR, Tracey BM *et al*. Secondary carnitine insufficiency in disorders of organic acid metabolism: modulation of acyl-CoA/CoA ratios by L-carnitine *in vivo*. *Biochem Soc Trans* 1983; **11**: 724.
60. Batshaw ML, Brusilow SW. Treatment of hyperammonemia coma caused by inborn errors of urea synthesis. *J Pediatr* 1980; **97**: 893.
61. Dasouki M, Buchanan D, Mercer N *et al*. 3-Hydroxy-3-methylglutaric aciduria: response to carnitine therapy and fat and leucine restriction. *J Inherit Metab Dis* 1987; **10**: 142.

THE LACTIC ACIDEMIAS AND MITOCHONDRIAL DISEASE

46.	Introduction to the lactic acidemias	337
47.	Pyruvate carboxylase deficiency	347
48.	Fructose-1,6-diphosphatase deficiency	354
49.	Deficiency of the pyruvate dehydrogenase complex	359
50.	Lactic acidemia and defective activity of pyruvate, 2-oxoglutarate, and branched chain oxoacid dehydrogenases	368
51.	Mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS)	374
52.	Myoclonic epilepsy and ragged red fiber (MERRF) disease	382
53.	Neurodegeneration, ataxia, and retinitis pigmentosa (NARP)	388
54.	Kearns-Sayre syndrome	393
55.	Pearson syndrome	398
56.	The mitochondrial DNA depletion syndromes: mitochondrial DNA polymerase deficiency	404

Introduction to the lactic acidemias

Work up of a patient with congenital lactic acidemia	338	Treatment	344
Clinical abnormalities	343	References	344
Pathogenesis	344		

The lactic acidemias constitute a large family of distinct disorders of metabolism. There are enlarging numbers of enzymatic deficiencies, and some disorders are now characterizable on the basis of the mutation in the DNA. This is especially the case in mitochondrial DNA, but mutations are increasingly being detected in nuclear DNA. Some patients have lactic acidemia secondary to another disorder, such as propionic acidemia ([Chapter 2](#)). On the other hand, there remain a considerable number of patients with lactic acidemia in whom a molecular explanation of the abnormal metabolism cannot be found, even with the most sophisticated studies available. Elucidating the cause and the most appropriate approach to therapy in a patient with lactic acidemia requires a systematic investigation.

In considering a patient for investigation of lactic acidemia, it is first necessary to establish that elevation of lactic acid in the blood is real. This may require a number of determinations even in patients with known disease. The most common reason for elevated concentration of lactic acid in blood is improper technique, the use of a tourniquet, or a real struggle in obtaining a sample. On the other hand, levels are variable even in patients with known disease. This is a function of the fact that lactic acid itself is situated some distance from most of the known defective enzymatic steps, particularly oxidative steps in the electron transport chain. The first step is the documentation of elevated levels of lactic acid, pyruvic acid, and/or alanine in the blood. It is important to be rigorous about methods of sampling, to draw blood that is flowing freely without a tourniquet. Our best results are often obtained in the course of studies in the clinical research center in which a catheter is placed in the vein to permit multiple sampling without the stresses of venepuncture. The concentration of lactate in the cerebrospinal fluid (CSF) may also be elevated. Increasingly, patients are encountered in whom the concentration of lactate in the CSF is elevated, whereas that of plasma is normal or only slightly or intermittently elevated [1].

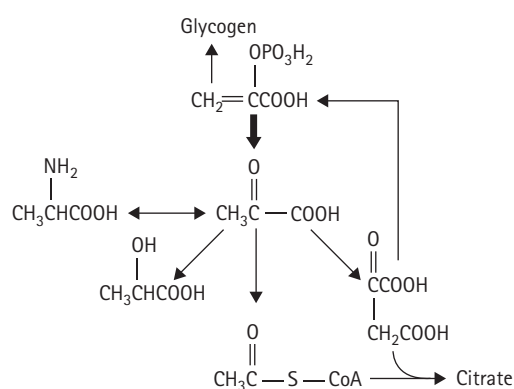


Figure 46.1 Pyruvate plays a central role in glycolysis and in oxidative metabolism. The pyruvate does not accumulate when its metabolism is blocked; it is converted to sinks or reservoirs of lactate and alanine.

The lactic acidemias are disorders of pyruvate metabolism. Concentrations of pyruvate are determined, but large elevations of pyruvate are seldom seen. Accumulating pyruvate is converted to lactate and alanine ([Figure 46.1](#)). Concentrations of alanine are not raised factitiously by problems of technique, but they too are variable in patients with known enzymatic defects.

The next step is to exclude the conditions that lead to secondary elevations in concentrations of lactic acid. A major group of patients are those with hypoxia, hypoventilation, shock, or hypoperfusion. These situations are seen in patients with sepsis, cardiac and pulmonary disease, hepatic disease, and severe anemia. Therefore, all of these conditions should be excluded before undertaking a metabolic work up for the elucidation of a lactic acidemia. Anaerobic exercise also produces lactic acidemia, but this is seldom an issue clinically, except in the patient who has just had convulsions.

Among patients with metabolic disease, lactic acidemia is often seen, particularly at times of acute illness, as a secondary complication of the underlying metabolic defect. These metabolic diseases include propionic acidemia (Chapter 2), methylmalonic acidemia (Chapter 3), isovaleric acidemia (Chapter 7), 3-hydroxy-3-methylglutaric aciduria (Chapter 45), and pyroglutamic aciduria. Lactic acidemia occurs in multiple carboxylase deficiency (Chapter 5) as a direct consequence of the defect in pyruvate carboxylase. Each of these conditions can be excluded by organic acid analysis [2–4]. Disorders of fatty acid oxidation (Chapters 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45) may also lead to acute elevations in the concentration of lactic acid. They are best excluded by assay of the acylcarnitines of the blood (Chapter 32) [5].

WORK UP OF A PATIENT WITH CONGENITAL LACTIC ACIDEMIA

The search for mutation in mitochondrial DNA may lead directly to the molecular diagnosis. We also determine the acylcarnitine profile by tandem mass spectrometry. This might already have been done to rule out a disorder of fatty acid oxidation, but we are increasingly referred patients who are known only to have lactic acidemia, and it is convenient to carry out both assessments. This is particularly important because patients with defects in the respiratory chain can have secondary alterations in fatty acid oxidation [6, 7]. In studies in which fibroblasts were labeled with deuterated hexadecanoic acid in the presence of carnitine, a variety of patterns were observed in patients with known electron transport deficits, including the C4 pattern mimicking short chain acylCoA dehydrogenase (SCAD) deficiency (Chapter 41) and the C6, C8, C10 pattern of medium chain acylCoA dehydrogenase (MCAD) deficiency (Chapter 38).

Examination of mitochondrial DNA should include a search for the common point mutations, as well as a Southern blot for deletions. This should yield definitive diagnoses in the case of mitochondrial encephalomyelopathy lactic acidemia and stroke-like episodes (MELAS) (Chapter 51), mitochondrial encephalomyelopathy and ragged red fibers (MERRF) (Chapter 52), neurodegeneration, ataxia, and retinitis pigmentosa (NARP) (Chapter 53), Kearns-Sayre syndrome (Chapter 54), and Pearson syndrome (Chapter 55).

Patients judged to have congenital lactic acidemia and not found to have an abnormality in mitochondrial DNA fall into two categories, those with defects in gluconeogenesis and those with defects in oxidation (Figure 46.2). It is important in the work up to distinguish clearly into which of the two categories each patient falls. The distinction may be useful in designing therapy, even in those patients in whom a molecular diagnosis remains elusive.

The central feature of this assessment (Figure 46.3) is to undertake a prolonged fast in which glucagon is given

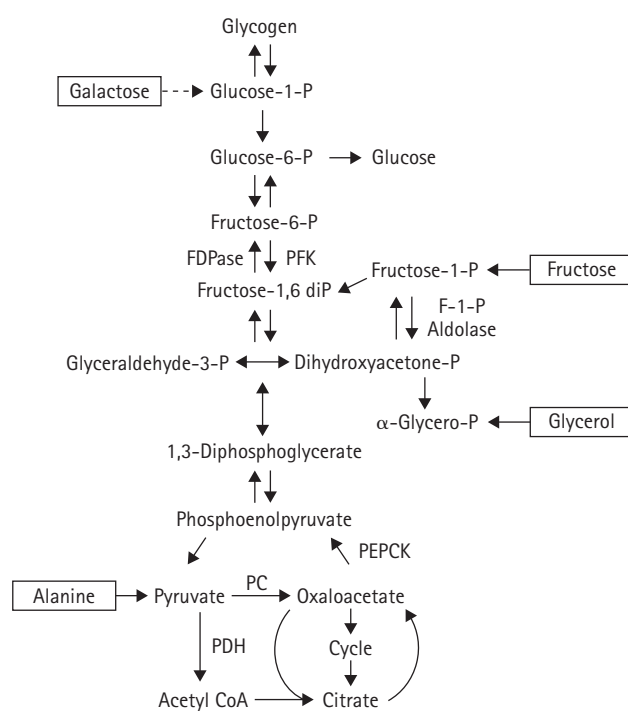


Figure 46.2 Pathways of metabolism for pyruvate through gluconeogenesis and oxidation. Boxes highlight compounds that have been used in tests to elucidate defects in gluconeogenesis.

early in order to deplete the liver of glycogen made from glucose. As the fast is continued for 18–24 hours, the body must carry out gluconeogenesis in order to maintain euglycemia. At the end of the fast, this is confirmed by another glucagon test. In the absence of gluconeogenesis, the blood concentration of glucose does not rise.

In this procedure, an intravenous catheter is inserted to facilitate the drawing of samples. Prior to the initiation of fasting, blood is obtained for glucose, lactate, pyruvate, and alanine. After 6 hours of fasting, 0.5 mg of glucagon is given intramuscularly, and the glucose response is determined at 15, 30, 45, 60, and 90 minutes. The response to glucagon should be a sizable increase in glucose, except in glycogenosis type I (Chapter 59). The fast is then continued for 24 hours if the patient remains euglycemic. The blood concentration of glucose is monitored by determination at the bedside and quantitative determinations are carried out at intervals and in the presence of an abnormal test or symptoms of hypoglycemia. If hypoglycemia develops at any time, the fast is concluded and glucagon given. The intravenous catheter ensures the prompt intravenous administration of glucose to restore normoglycemia. Concentrations of lactic and pyruvic acids and alanine are obtained at the end of the fast prior to the administration of glucagon. In a hypoglycemic patient, levels of insulin, growth hormone, and glucagon are also obtained if this information is not available from prior testing.

In a patient who fails the fasting test and appears to

Algorithmic workup of patient with lactic acidemia

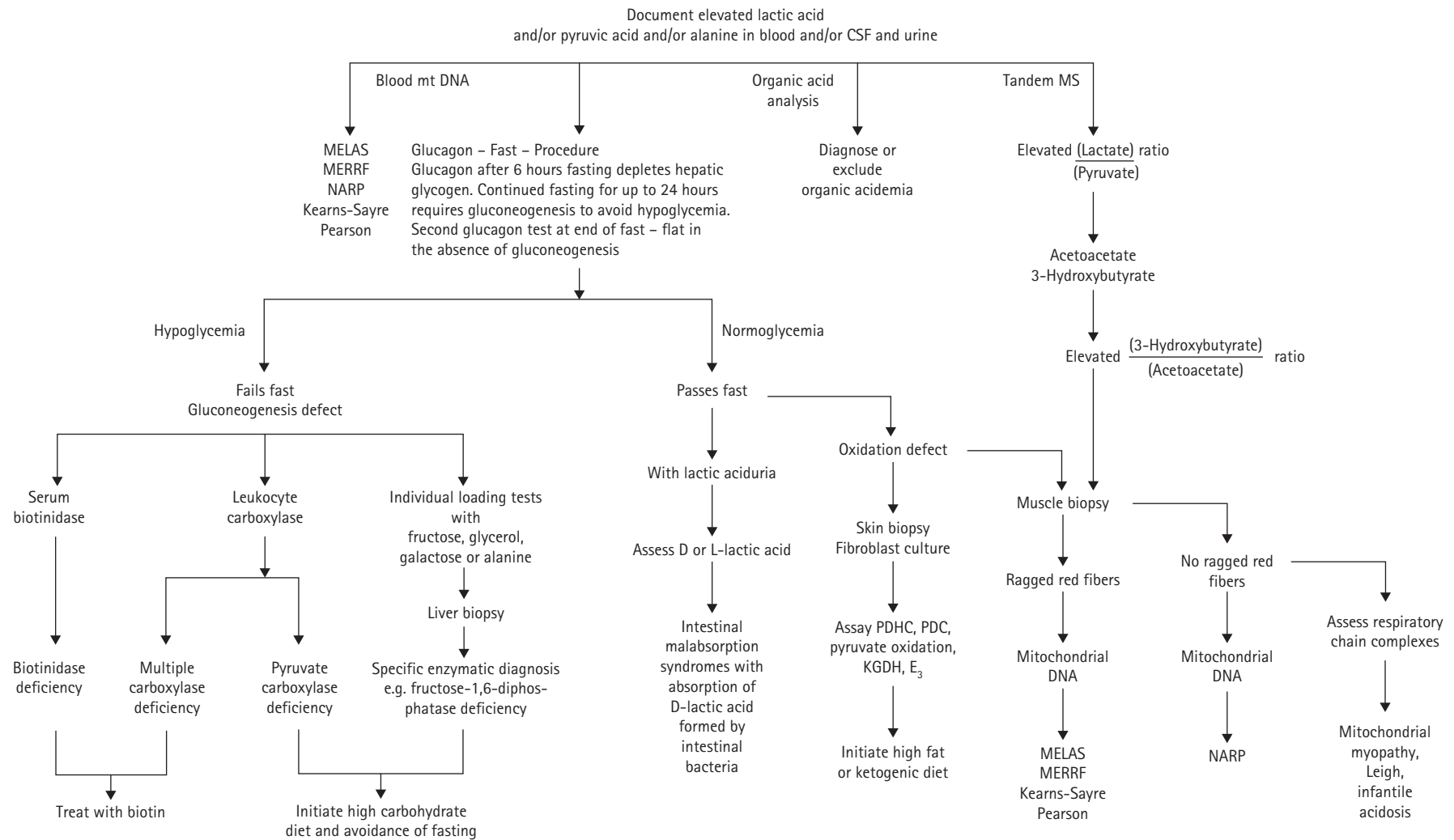


Figure 46.3 Algorithm for decision-making in congenital lactic acidemia. CSF, cerebrospinal fluid; E₃, lipoamide dehydrogenase; KGDH, 2-oxoglutarate dehydrogenase; PDC, pyruvate decarboxylase; PDHC, pyruvate dehydrogenase complex.

have a defect in gluconeogenesis, it is convenient to assay biotinidase [4] in serum and carboxylases in leukocytes or fibroblasts; these procedures are noninvasive and provide a rapid answer to the diagnosis. Most patients with disorders of gluconeogenesis in whom these two procedures do not provide the diagnosis require liver biopsy for definitive enzyme assay. Information as to the area of the defect may be obtained by loading tests, for instance with fructose, alanine, or glycerol in fructose-1,6-diphosphatase deficiency (Chapter 48) [8, 9]. Following glycerol or fructose, phosphate should also be measured because it decreases sharply in patients with a block at this level. Concentrations of uric acid may increase. Loading with galactose should provide a positive control except in a patient with glucose-6-phosphatase deficiency. Each compound is given by mouth as a 20 percent solution 6–12 hours postprandially in a dose of 1 g/kg.

Most patients who pass the fasting test have defects in oxidation of pyruvate. A small number has essentially factitious lactic acidemia with lactic aciduria in which D-lactic acid is formed by intestinal bacteria and then absorbed. L-lactic acid and L-alanine are actually dextrorotatory in the polarimeter, but the nomenclature is employed to indicate their structural similarity to L-glyceraldehyde (Figure 46.4). D-lactic acid and D-amino acids are bacterial components. N-Acetylmuramic acid, a compound of D-lactic acid and N-acetylglucosamine, is a component of the mucopeptides of bacterial cell walls. D-lactic aciduria is usually seen in patients with malabsorption syndromes, as well as the short bowel syndrome and necrotizing enterocolitis [10, 11]. This lactic acid accumulation can even lead to systemic acidosis and coma. A course of treatment with oral neomycin or metronidazole may resolve this problem, as may testing for lactate with an enzymatic assay specific for L-lactic or D-lactic acid. Factitious lactic acidemia and/or lactic aciduria may also occur in the neonatal intensive care unit or elsewhere when glucose is infused in amounts in excess

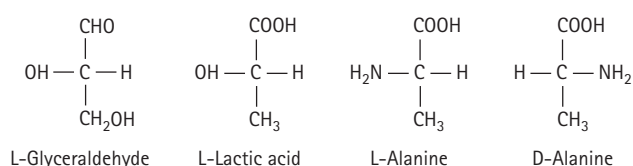


Figure 46.4 Structure of L-lactic acid and related compounds.

of the capacity of the infant to utilize it [12].

In patients who pass the test and are judged to have defective oxidation of pyruvate, we carry out skin biopsies for fibroblast culture and usually initiate therapy with a diet high in fat and low in carbohydrate while the culture is being established. Once the fibroblasts are available, they are assayed for defects in the pyruvate dehydrogenase complex (PDHC) and in its first enzyme, pyruvate decarboxylase (E1). E1 has an α - and a β -subunit. Defects in E1 α can be tested for by mutational analysis (Chapter 49). The E2 transacetylase protein can be tested for by Western blot analysis and some mutations have been defined. In patients with defects in this PDHC system, it is also useful to measure the activity of α -ketoglutarate dehydrogenase and lipoamide dehydrogenase (E3).

Muscle biopsy with histology and studies of electromyography and nerve conduction are employed to elucidate patients with myopathy, or abnormalities in mitochondrial structure. Ragged red fibers are frequently seen in disorders of mitochondrial DNA. Muscle may be used as a source for analysis of mutation in the DNA. There are patients in whom analysis of muscle reveals the heteroplasmy, while the blood does not. Fresh muscle obtained by open biopsy permits the best assessment of the activity of the complexes of the electron transport chain (Figures 46.5 and 46.6). In some laboratories, these assays are done on frozen muscle or on freshly isolated platelets. In addition, patients have been reported [13] in whom defective activities of the electron transport chain has been documented by assay of

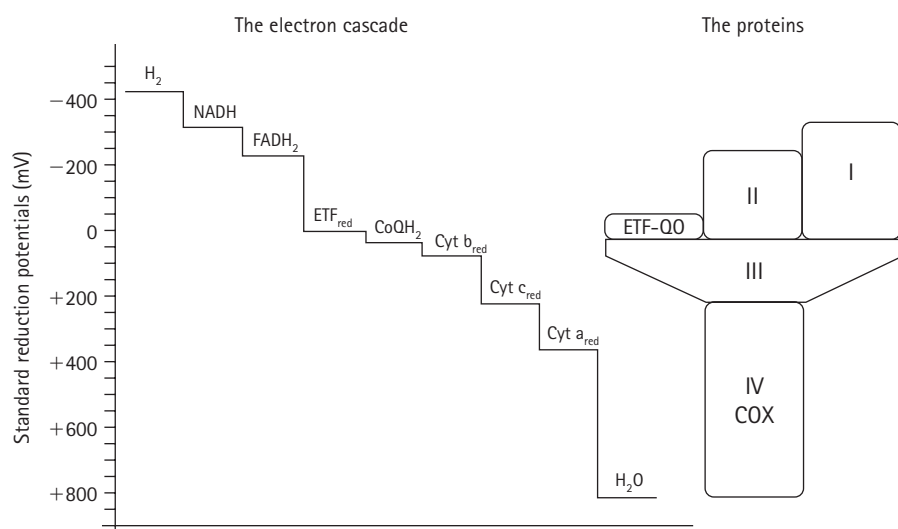


Figure 46.5 The mitochondrial electron transport chain. Lactic acidemia and an elevated lactate: pyruvate ratio may occur with any defect that interferes with the utilization of NADH. CoQ10, coenzyme Q10; Cyt, cytochrome; NAD, nicotinamide adenine dinucleotide; NADH, reduced NAD (FADH₂ its flavoprotein).

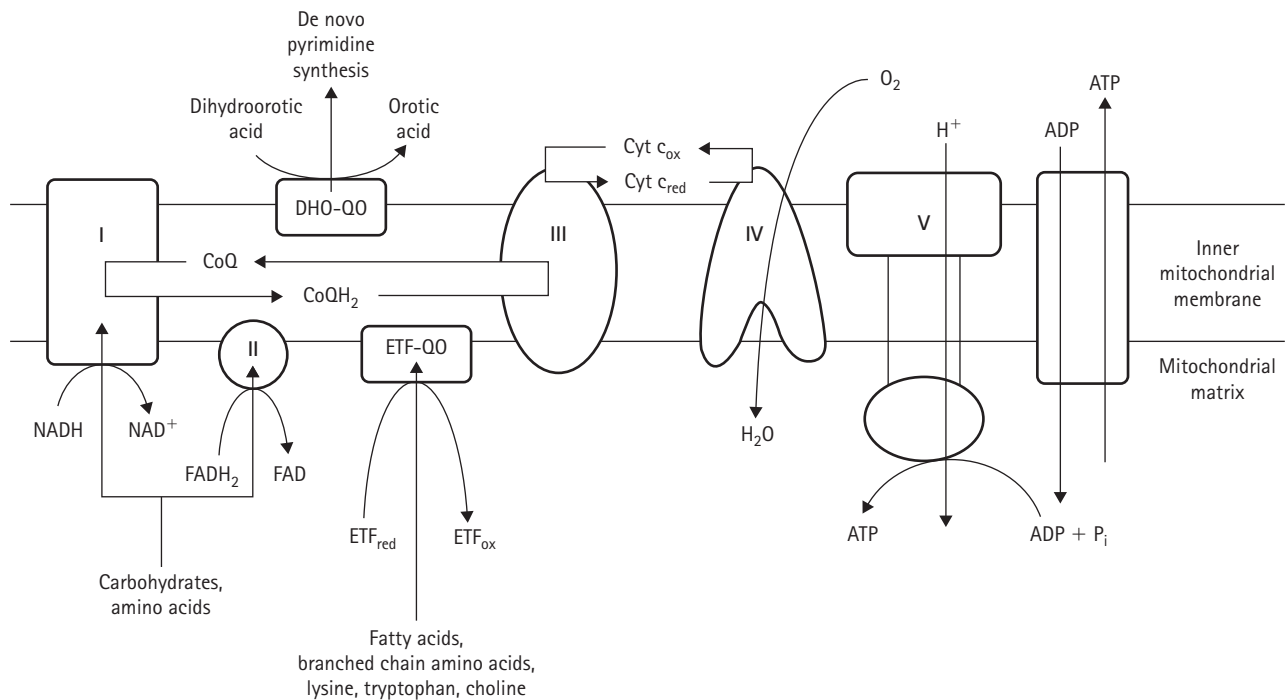


Figure 46.6 The electron transport chain.

biopsied liver, even in patients in whom there have been no hepatic mechanisms of disease.

The lactate to pyruvate ratio in the blood is usually elevated in electron transport abnormalities, and this abnormality may trigger a muscle biopsy ([Figure 46.3](#)). Ragged red fibers suggest analysis of mitochondrial DNA, while their absence suggests assay for a nuclear encoded defect in the respiratory chain. These complexes I to V are a mixture of mitochondrial and nuclear encoded proteins.

Energy conversion takes place in mitochondria in which the exergonic oxidation/reduction reactions of the electron transport chain, as in chloroplasts and bacteria, are coupled to the endergonic synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate [14]. The electron flow generates a proton motive force. The ATP synthase is a large asymmetric enzyme complex of an F_0F_1 structure, in which the F_0 is a hydrophobic, membrane-embedded unit that serves

as a proton channel, while the F_1 contains the nucleotide binding sites and catalytic sites for ATP synthesis. When solubilized and uncoupled from its F_0 energy source, the F_1 is capable of ATP hydrolysis, and this is why it is referred to as an ATPase.

The oxidative phosphorylation system is embedded in the lipid bilayer of the mitochondrial inner membrane. In addition to the five multiprotein enzyme complexes there are two electron carriers – coenzyme Q and cytochrome C. The ATP generated by oxidative phosphorylation may be used in the mitochondrion or transported out by the adenine nucleotide transporter for other cellular purposes. Each of the complexes of the electron transport chain except complex II contains proteins encoded by the mitochondrial DNA, as well as proteins encoded by nuclear DNA ([Table 46.1](#)). Mitochondrial DNA and its mutations are maternally inherited; nuclear DNA mutations in this system are inherited autosomally.

Table 46.1 The genetic determination of the proteins of the electron transport chain

Complex	Name	Mitochondrial genes	Nuclear genes	Total
I	NADH: ubiquinone oxidoreductase	7	>44	>51
II	Succinate: ubiquinone oxidoreductase	0	4	4
III	Cytochrome bc	1	>10	>11
IV	Cytochrome c oxidase	3	10	13
V	ATP Synthase	2	14	16
Total		13	>82	>95

In addition to mutations in the genes coding for proteins of the electron transport chain, there are mutations causing mitochondrial disease in proteins involved in the assembly and maintenance of mitochondrial proteins. Increasingly, mutations are being found in the nuclear encoded functions of oxidative phosphorylation. A majority, so far, have been in complex I [15–19]. Some of these mutations, which disrupt respiratory chain function, do not produce lactic acidemia. Knowledge of the molecular mutation in a family permits accurate prenatal diagnosis, whereas biochemical methods have frequently been in error [20]. Among mutations in genes for proteins involved in the assembly of respiratory chain proteins SURF1, which functions in assembly of complex IV, is probably the best studied [21–23] and produces a severe clinical Leigh syndrome.

Molecular chaperones are required for the assembly of the catalytic F_1 component of the mitochondrial ATP synthase, and probably for many other proteins involved in mitochondrial function. Those for F_1 have been well studied in yeast and mutations in *Saccharomyces* are known. The human genes for orthologs are known. Their study may reveal novel mechanisms of mitochondrial disease.

Mitochondrial proteins synthesized in the cytosol must be transported into the mitochondria. Defects in the transport proteins could provide another novel mechanism of the pathogenesis of mitochondrial disease. Two of these protein complexes, translocation outer mitochondrial membrane (TOMM) and translocation inner mitochondrial membrane (TIMM), have been extensively studied in yeast in which the genes have been characterized. The human gene encoding an ortholog of TOMM 20 has been identified [24], and the human genome project has made the genes for other orthologs available. A search for abnormalities causing human disease is under way. Among the transporters of the mitochondria is an outer membrane transporter known as voltage-dependent ion channel (VDAC) and also known as mitochondrial porin, because it forms a pore, opening the membrane for anions like phosphate, chloride, and adenine nucleotides at low transmembrane voltage; at high voltage, it forms a channel for cations and uncharged molecules. A deficiency in VDAC has been reported in Western blot studies [25] in a patient with impaired myopathy and impaired oxidation of pyruvate in mitochondria of muscle. Lactate was elevated only mildly after 2 g/kg of glucose.

Concentrations of L-lactic acid in body fluids are a function of the concentrations of pyruvate and the ratio of NAD^+ to NADH. This may be expressed as:

$$K = \frac{\text{Lactate} (NAD^+)}{(\text{Pyruvate})(NADH)(H^+)}$$

When $NAD/NADH$ ratios are constant, changes in lactate concentration are a function only of the metabolism of pyruvate, but the ratio of NAD to $NADH$ reflects the

oxidative state of the cell [26]. Hypoxia increases NADH, while oxidation regenerates NAD. The normal ratio of $NADH$ to NAD in aerobic tissues is about 10 to 1, and the ratio of lactate to pyruvate does not normally exceed 15 [27]. Elevation of the cytosolic ratio of lactate to pyruvate, often considered to be above 25, indicates a disorder of oxidative phosphorylation. An elevation of the lactate to pyruvate ratio in the absence of elevation of the 3-hydroxybutyrate to acetoacetate ratio indicates a severe deficiency of pyruvate carboxylate (Chapter 47). The ratio of 3-hydroxybutyrate to acetoacetate may more closely reflect the redox state of the mitochondria. Conditions in which there is an excess of $NADH$ will cause an elevated lactate to pyruvate or 3-hydroxybutyrate to acetoacetate ratio. When the electron transport chain is not functioning well, $NADH$ cannot be converted to NAD and hence ATP is not produced. Compensation by conversion of pyruvate to lactate regenerates some NAD .

$NADH$ is the fuel of the cytochrome chain (Figures 46.5 and 46.6). Therefore, an abnormality in a cytochrome such as cytochrome oxidase deficiency [28–30] will lead to abnormalities in these ratios because of diminished utilization of $NADH$. Defects in the respiratory chain may be demonstrated in cultured fibroblasts, as well as in muscle [28, 29, 31–39], but a group of patients has been described [34–38] in which defects in the respiratory chain in muscle were not demonstrable in fibroblasts. In a patient with fatal neonatal lactic acidosis [39], the ratio of lactate to pyruvate was 136:1. The ratio of 3-hydroxybutyrate to acetoacetate was 42:1. In fibroblasts, the conversion of $1\text{-}^{14}\text{C}$ -pyruvate and glutamate to $^{14}\text{CO}_2$ was defective, and when the cells were incubated with glucose, an elevated lactate to pyruvate ratio of 72:1 was observed. In control cells, the ratio was 20:1. The ratio has also been used to indicate a defect in pyruvate dehydrogenase where it is expected to be low or normal. It has been established that in classifying patients as having a respiratory chain dysfunction versus pyruvate dehydrogenase, the ratio of lactate to pyruvate is only useful when the level of lactate is high [40]. The ratio in the CSF may be used, as well as that of the blood.

We regularly employ a modified oral glucose tolerance test in which the standard 1.75 g/kg is monitored by assessment of concentrations of lactate, pyruvate, and alanine which may rise as much as four-fold over control levels in a patient with PDHC deficiency. This evidence of glucose intolerance may be useful in designing therapy that avoids carbohydrate and substitutes fat, as well in monitoring the efficacy of therapeutic interventions. Fructose loading has been used in assessing *in vivo* pyruvate dehydrogenase activity and its activation. After a 12–24-hour fast, blood samples are drawn for lactate, pyruvate, glucose, and insulin before and 45 minutes after an oral load of 1 g/kg of fructose. The test is then repeated after an oral glucose load. The rise in blood pyruvate and lactate was reported to be almost twice as great in the fasted as in the postglucose state, suggesting the conversion of pyruvate

dehydrogenase to its active form by glucose feeding. Studies have not been reported on actual patients with problems with pyruvate dehydrogenase, and we have not found this to be especially useful. Empirically, an occasional patient has responded to fructose with a marked increase in lactic acid, but as a group the patients with mitochondrial disease have not been reliably distinguishable from control in their response to fructose.

A postprandial rise in lactate (greater than two-fold) occurs in pyruvate dehydrogenase deficiency and also in glycogen storage diseases types 0, III, and VI/IX. In primary defects of the respiratory chain, the redox state may become more abnormal; in addition, there may even be a rise of total ketone bodies (paradoxical ketonemia). A postprandial fall of lactate occurs in glycogen storage disease type I and defects of gluconeogenesis. In glycogen synthase deficiency, concentrations of lactate and alanine are low when the patient is hypoglycemic, but feeding or a glucose tolerance test leads to elevated amounts of lactate, as well as hypoglycemia.

The urinary lactate may be useful in diagnosis [2] and the lactate to creatinine ratio has been employed for this purpose. The normal range is from 0.028 to 0.22. A schematic approach to the use of the blood and urinary lactate in differential diagnosis is shown in Figure 46.7. In congenital lactic acidosis, both the blood and urinary lactate should be elevated. However, each may be quite variable in any individual patient. Therefore, it is prudent in a patient suspected of having lactic acidosis to carry out a number of assays at various times.

CLINICAL ABNORMALITIES

The clinical manifestations of the congenital lactic acidemias have many similarities regardless of the specific causes. There are a number of distinct clinical syndromes

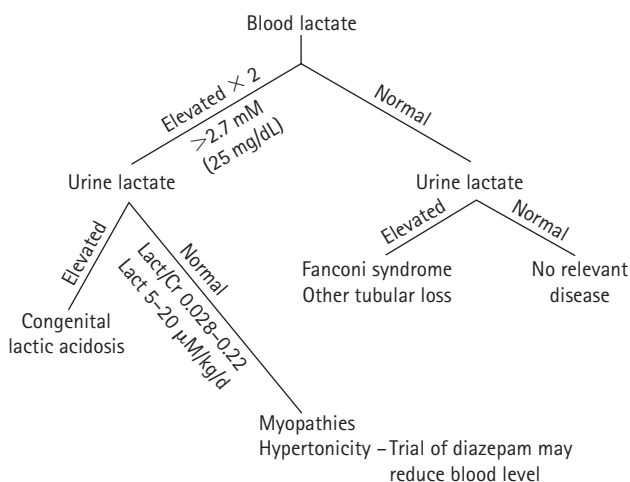


Figure 46.7 Assessment of lactate in urine as an aid to differential diagnosis of lactic acidemia.

[41, 42]. One of them is acute metabolic acidosis, usually neonatal or infantile. Congenital lactic acidosis was first described by Hartman and colleagues in 1962 [43] in patients with this clinical picture. A number of patients have been reported [44, 45] in whom the picture is that of recurrent episodes of acidosis with hyperventilation, any one of which may lead to coma and death. Attacks of unexplained vomiting may herald onset. Some patients have acute symptomatic hypoglycemia. This may lead to convulsive seizures, but seizures may also occur in the absence of hypoglycemia. Any patient with chronic lactic acidosis may develop pulmonary hypertension and this may be the cause of death.

Another presentation, especially in patients in later infancy or childhood, is with ataxia. This may be episodic or chronic. Episodes may be precipitated by stress, such as an intercurrent infection. Between attacks, the patient may be clumsy. Often, there is associated episodic neurologic degeneration.

Another type of presentation is with Leigh syndrome, or subacute necrotizing encephalomyelopathy [46–48]. This was essentially a histopathologic diagnosis, usually made at autopsy in an ultimately fatal disease. The neuropathologic picture resembles Wernicke encephalopathy in the basal ganglia, brain stem, and cerebellum, but in contradistinction to the picture in Wernicke disease, the mammillary bodies are usually spared. Spongiform degeneration is seen, as are increased vascularity and glial proliferation. Computed tomography

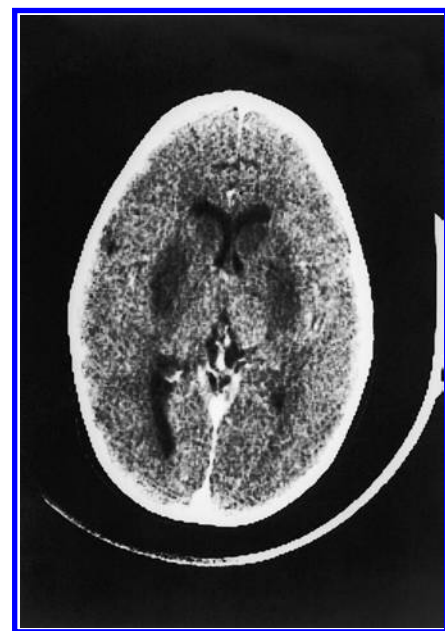


Figure 46.8 Computed tomography scan of the head of a patient with lactic acidemia and Leigh encephalopathy, illustrating advanced degenerative changes in the brain stem, as well as the basal ganglia.

(CT) or magnetic resonance imaging (MRI) scans now provide the neuroimaging counterpart of the histology with hypodensity in the caudate and putamen (Figure 46.8) [48, 49]. Ultimately, the patient develops spasticity often with Babinski signs. Seizures occur in about a third of such patients. Blindness may supervene. There may be retinal pigment epithelium changes. Late in the course, deep tendon reflexes may be absent. Tracheostomy and artificial ventilation may be required. This picture of Leigh encephalomyelopathy is clearly independent of etiology. It has been described with deficiency of the pyruvate dehydrogenase complex [50] and in a patient with defective activation of the pyruvate dehydrogenase complex because of deficiency of pyruvate dehydrogenase phosphatase [47]. It may be seen in patients with NARP mutation.

We have studied a small subgroup of patients with severe deficiency of the pyruvate dehydrogenase complex (PDHC) in whom there was a recognizable syndrome of dysmorphic features (Chapter 49). A sibling had a similar clinical appearance. Our first patient appeared to be cortically blind in infancy. Another patient had gross abnormalities in the morphogenesis of the brain.

Another group of patients has the picture of a metabolic myopathy or ophthalmoplegia [50–54]. In some, there was associated neurodeafness or early cataracts. Two siblings had sideroblastic anemia. Many of these patients have had muscle weakness, usually of insidious onset, in a pattern of a proximal muscular dystrophy. Ptosis, facial muscle weakness, and cardiomyopathy have also been seen. Electron microscopy may reveal large mitochondria, often with a bizarre appearance. Some patients have ragged, red fiber changes in the histology of muscle. Many of these have had abnormalities in mitochondrial DNA.

PATHOGENESIS

Patients with evidence of defective activity of many mitochondrial enzymes exemplify the complex nature of the pathogenesis of mitochondrial disease. For instance, mitochondrial DNA depletion (Chapter 56) leads to defective activity of most of the complexes of the electron transport chain. Similarly, patients were reported [55] in whom there was defective activity of pyruvate and 2-oxoglutarate dehydrogenase complexes, NADH cytochrome c reductase, succinate dehydrogenase, and succinate cytochrome reductase. This fatal disease in three siblings was shown by microcell-mediated transfer to a panel of mouse–human hybrids to be under control of a nuclear gene on chromosome 2 at 2p13–14.

Metabolic coupling has been observed in the relationship between glial cells and neurons involving the metabolism of lactate [56]. Astrocytes appear to be the main site of glucose uptake during neuronal activity. Glucose is then processed glycolytically in these astrocytes, which then release lactate which is the metabolic substrate used by neurons.

TREATMENT

Acute treatment of metabolic acidosis may require large amounts of sodium bicarbonate. Sodium citrate may be ineffective in a patient with an oxidative defect because citric acid cycle function is impaired. Chronic treatment may be undertaken in patients with lactic acidemia prior to the establishment of a definitive diagnosis, provided enough is known about the underlying pathophysiology. Thus, patients with disorders of gluconeogenesis should avoid fasting and require intravenous glucose during intercurrent illnesses in which the oral route is not available, as in the vomiting patient. A diet high in carbohydrate is therapeutic and cornstarch supplementation may be helpful.

Patients with disorders of oxidation are, in contrast, often glucose-sensitive and respond with reduction in lactate concentrations to a diet high in fat [57]. Diets employed contain 50 percent or more of the calories from fat. They do not have to be ketogenic.

Lactate levels can be lowered in some patients by the administration of dichloroacetate (DCA), regardless of the cause. It is not generally recommended in disorders of gluconeogenesis, because DCA can itself produce hypoglycemia. Its use has been employed experimentally in a variety of other lactic acidemic conditions. Dichloroacetate activates the PDHC by inhibiting PDH kinase. *In vivo*, this compound reduces concentrations of lactate, pyruvate, and alanine [58, 59], and increases the percentage of the active form of PDHC in brain, liver, and muscle. It has been used to treat congenital lactic acidosis [60–62], and levels of lactic acid have been improved. Neurologic improvement has been elusive in most patients reported, but there have been some successes. Peripheral neuropathy can be expected to worsen with DCA, and some patients develop peripheral neuropathy [63].

REFERENCES

1. Brown GK, Haan EA, Kirby DM *et al*. 'Cerebral' lactic acidosis: defects in pyruvate metabolism with profound brain damage and minimal systemic acidosis. *Eur J Pediatr* 1988; **147**: 10.
2. Chalmers RA. Organic acids in urine of patients with congenital lactic acidoses: an aid to differential diagnosis. *J Inherit Metab Dis* 1984; **7**: 79.
3. Hoffmann G, Aramaki S, Blum-Hoffmann E *et al*. Quantitative analysis for organic acids in biological samples: batch isolation followed by gas chromatographic-mass spectrometric analysis. *Clin Chem* 1989; **35**: 587.
4. Thuy LP, Zielinska B, Zammarchi E *et al*. Multiple carboxylase deficiency due to deficiency of biotinidase. *J Neurogenet* 1986; **3**: 357.
5. Vreken P, van Lint AEM, Bootsma AH *et al*. Rapid diagnosis of organic acidemias and fatty acid oxidation defects by quantitative electrospray tandem-MS acyl-carnitine analysis in plasma. In: Quant PA, Eaton S (eds). *Current Views of Fatty Acid*

- Oxidation and Ketogenesis: From Organelles to Point Mutations*. New York: Plenum Publishers, Kluwer Academic, 1999: 327.
6. Sim KG, Carpenter K, Hammond J *et al*. Acylcarnitine profiles in fibroblasts from patients with respiratory chain defects can resemble those from patients with mitochondrial fatty acid β -oxidation disorders. *Metabolism* 2002; **51**: 366.
 7. Enns GM, Bennett MJ, Hoppel CL *et al*. Mitochondrial respiratory chain complex I deficiency with clinical and biochemical features of long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *J Pediatr* 2000; **136**: 251.
 8. Baker L, Winegrad AI. Fasting hypoglycaemia and metabolic acidosis associated with deficiency of hepatic fructose-16-diphosphatase activity. *Lancet* 1970; **2**: 13.
 9. Pagliara AS, Karl IE, Keating JP *et al*. Hepatic fructose-66-diphosphatase deficiency: a cause of lactic acidosis and hypoglycemia in infancy. *J Clin Invest* 1972; **51**: 2115.
 10. Perlmutter DH, Boyle JT, Campos JM *et al*. D-lactic acidosis in children: an unusual metabolic complication of small bowel resection. *J Pediatr* 1983; **102**: 234.
 11. Garcia J, Smith FR, Cucinell SA. Urinary D-lactate excretion in infants with necrotizing enterocolitis. *J Pediatr* 1984; **104**: 268.
 12. Chalmers RA, Lawson AM. *Organic Acids in Man. The Analytical Chemistry Biochemistry and Diagnosis of the Organic Acidurias*. London: Chapman and Hall, 1982.
 13. Garcia-Cazorla A, De Lonlay P, Rustin P *et al*. Mitochondrial respiratory chain deficiencies expressing the enzymatic deficiency in the hepatic tissue: a study of 31 patients. *Pediatrics* 2006; **149**: 401.
 14. Abrahams JP, Leslie AGW, Lutter R, Walker JE. Structure at 28 Å resolution of F1-ATPase from bovine heart mitochondria. *Nature* 1994; **370**: 621.
 15. Smeitink J, van den Heuvel L, DiMauro S. The genetics and pathology of oxidative phosphorylation. *Nat Rev Genet* 2001; **2**: 342.
 16. van den Huevel L, Ruitenbeek W, Smeets R *et al*. Demonstration of a new pathogenic mutation in human complex I deficiency: A 5-bp duplication in the nuclear gene encoding the 18-kD (AODQ) subunit. *Am J Hum Genet* 1998; **62**: 262.
 17. Triepels RH, van den Huevel LP, Loeffen JL *et al*. Leigh syndrome associated with a mutation in the NDUFS7 (PSST) nuclear encoded subunit of complex I. *Ann Neurol* 1999; **45**: 787.
 18. Loeffen J, Smeitink J, Triepels R *et al*. The first nuclear-encoded complex I mutation in a patient with Leigh syndrome. *Am J Hum Genet* 1998; **63**: 1598.
 19. Schuelke M, Smeitink J, Mariman E *et al*. Mutant NDUFV1 subunit of mitochondrial complex I causes leukodystrophy and myoclonic epilepsy. *Nat Genet* 1999; **21**: 260.
 20. Schuelke M, Detjen A, van den Heuvel L *et al*. New nuclear encoded mitochondrial mutation illustrates pitfalls in prenatal diagnosis by biochemical methods. *Clin Chem* 2002; **48**: 772.
 21. Lee N, Morin C, Mitchell G, Robinson BH. Saguenay Lac Saint Jean cytochrome oxidase deficiency: sequence analysis of nuclear encoded COX subunits chromosomal localization and a sequence anomaly in subunit Vic. *Biochim Biophys Acta* 1998; **1406**: 1.
 22. Zhu Z, Yao J, Johns T *et al*. SURF1 encoding a factor involved in the biogenesis of cytochrome c oxidase is mutated in Leigh syndrome. *Nat Genet* 1998; **20**: 337.
 23. Tiranti V, Hoernagel K, Carozzo R *et al*. Mutations of SURF-1 in Leigh disease associated with cytochrome c oxidase deficiency. *Am J Hum Genet* 1998; **63**: 1609.
 24. Hernandez JM, Giner P, Hernandez-Yago J. Gene structure of the human mitochondrial outer membrane receptor Tom20 and evolutionary study of its family of processed pseudogenes. *Gene* 1999; **239**: 2283.
 25. Huizing M, Ruitenbeek W, Thinnies FP *et al*. Deficiency of the voltage-dependent anion channel: a novel cause of mitochondriopathy. *Pediatr Res* 1996; **39**: 760.
 26. Huckabee WE. Relationship of pyruvate and lactate during anerobic metabolism. I. Effects of infusion of pyruvate or glucose and hyperventilation. *J Clin Invest* 1958; **37**: 244.
 27. Cohen RD. Disorders of lactic acid metabolism. *Clin Endocrinol Metabol* 1967; **5**: 613.
 28. Heiman-Patterson TD, Bonilla E, DiMauro S *et al*. Cytochrome-c-oxidase deficiency in floppy infant. *Neurology* 1982; **32**: 898.
 29. Willems JL, Monnens LAH, Trijbels JMF *et al*. Leigh's encephalomyelopathy in a patient with cytochrome c oxidase deficiency in muscle tissue. *Pediatrics* 1977; **60**: 850.
 30. Lee N, Daly MJ, Delmonte T *et al*. A genome-wide linkage-disequilibrium scan localizes the Saguenay-Lac-Saint-Jean cytochrome oxidase deficiency to 2p16. *Am J Hum Genet* 2001; **68**: 397.
 31. DiMauro S, Mendell JR, Sahenk Z *et al*. Fatal infantile mitochondrial myopathy and renal dysfunction due to cytochrome-c oxidase deficiency. *Neurology* 1980; **32**: 795.
 32. VanBiervliet JPM, Bruinvis L, Ketting D *et al*. Hereditary mitochondrial myopathy with lactic acidemia, a DeToni-Fanconi-Debre syndrome and a defective respiratory chain in voluntary striated muscles. *Pediatr Res* 1977; **11**: 1088.
 33. Miyabayashi S, Nariswar K, Tada K *et al*. Two siblings with cytochrome-c oxidase deficiency. *J Inher Metab Dis* 1983; **6**: 121.
 34. Morgan-Hughes JA, Daveniza P, Kahn SN *et al*. A mitochondrial myopathy characterized by a deficiency of reducible cytochrome b. *Brain* 1977; **100**: 617.
 35. Morgan-Hughes JA, Darveniza P, Landon DN *et al*. A mitochondrial myopathy with deficiency of respiratory chain NADH-CoQ reductase activity. *J Neurol Sci* 1979; **43**: 27.
 36. Spiro AJ, Moore CE, Pimeas JW *et al*. A cytochrome related inherited disorder of the nervous system and muscle. *Arch Neurol* 1970; **23**: 103.
 37. Moreadith RW, Batshaw ML, Ohnishi T *et al*. Deficiencies of the iron-sulfur clusters of mitochondrial reduced nicotinamide-adenosine dinucleotide-ubiquinone oxidoreductase (complex I) in an infant with congenital lactic acidosis. *J Clin Invest* 1984; **74**: 685.
 38. Clark JB, Heyes DJ, Buyrne E, Morgan-Hughes JA. Mitochondrial myopathies defects in mitochondrial metabolism in human skeletal muscle. *Biochem Soc Trans* 1983; **11**: 626.
 39. Robinson BH, McKay N, Toodyer P, Lancaster G. Defective intramitochondrial NADH oxidation in skin fibroblasts from an

- infant with fatal neonatal lactic acidemia. *Am J Hum Genet* 1985; **37**: 938.
40. Debray FG, Mitchell GA, Allard P *et al*. Diagnostic accuracy of blood lactate-to pyruvate molar ratio in the differential diagnosis of congenital lactic acidosis. *Clin Chem* 2007; **53**: 916.
 41. Robinson BH, Taylor J, Sherwood WG. The genetic heterogeneity of lactic acidosis: occurrence of recognizable inborn errors of metabolism in a pediatric population with lactic acidosis. *Pediatr Res* 1980; **14**: 956.
 42. Munnich A, Rotig A, Cormier-Daire V, Rustin P. Clinical presentation of respiratory chain deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic and Molecular Bases of Inherited Disease*. New York: McGraw Hill, 2001: 2261.
 43. Hartman Sr AF, Wohltmann HJ, Puckerson ML, Wesley ME. Lactic acidosis: studies of a child with a serious congenital deviation. *J Pediatr* 1962; **61**: 165.
 44. Israel S, Haworth JC, Gourley B, Ford JD. Chronic acidosis due to an error in lactate and pyruvate metabolism. *Pediatrics* 1964; **34**: 346.
 45. Skrede S, Stromme JH, Stokke O *et al*. Fatal congenital lactic acidosis in two siblings. II. Biochemical studies *in vivo* and *in vitro*. *Acta Paediatr Scand* 1971; **60**: 138.
 46. Pincus JH. Subacute necrotizing encephalomyelopathy (Leigh's disease): a consideration of clinical features and etiology. *Dev Med Child Neurol* 1972; **14**: 87.
 47. DeVivo DC, Haymond MW, Obert KA *et al*. Defective activation of the pyruvate dehydrogenase complex in subacute necrotizing encephalomyelopathy (Leigh disease). *Ann Neurol* 1979; **6**: 483.
 48. Schwarz WJ, Hutchinson HT, Berg BO. Computerized tomography in subacute necrotizing encephalomyelopathy (Leigh disease). *Ann Neurol* 1981; **10**: 268.
 49. Chi JG, Yoo HW, Chang KY *et al*. Leigh's subacute necrotizing encephalomyelopathy: possible diagnosis by CT scan. *Neuroradiology* 1981; **22**: 141.
 50. Evans OB. Episodic weakness in pyruvate decarboxylase deficiency. *J Pediatr* 1984; **105**: 961.
 51. Johnston K, Newth CJL, Sheu K-FR *et al*. Central hypoventilation syndrome in pyruvate dehydrogenase complex deficiency. *Pediatrics* 1984; **74**: 1034.
 52. Hackett TN Jr, Bray PR, Ziter FA *et al*. A metabolic myopathy associated with chronic lactic acidemia growth failure and nerve deafness. *J Pediatr* 1973; **83**: 426.
 53. VanWijngaarden GK, Bethlem J, Meijer AEFH *et al*. Skeletal muscle disease with abnormal mitochondria. *Brain* 1967; **90**: 577.
 54. Shapira Y, Cederbaum SD, Cancilla PA *et al*. Familial poliodystrophy mitochondrial myopathy and lactate acidemia. *Neurology* 1975; **25**: 614.
 55. Seyda A, Newbold RF, Hudson TJ *et al*. A novel syndrome affecting multiple mitochondrial functions located by microcell-mediated transfer to chromosome 2p14-2p13. *Am J Hum Genet* 2001; **68**: 386.
 56. Tsacopoulos M, Magistretti PJ. Metabolic coupling between glia and neurons. *J Neuroscience* 1996; **46**: 877.
 57. Falk RE, Cederbaum SD, Blass JP *et al*. Ketogenic diet in the management of pyruvate dehydrogenase deficiency. *Pediatrics* 1976; **58**: 713.
 58. Kuroda Y, Toshim K, Watanabe T *et al*. Effects of dichloroacetate on pyruvate metabolism on rat brain *in vivo*. *Pediatr Res* 1984; **18**: 936.
 59. Stacpoole PW, Moore GW, Kornhauser DM. Metabolic effects of dichloroacetate in patients with diabetes mellitus and hyperlipoproteinemia. *N Engl J Med* 1978; **298**: 526.
 60. Robinson BH, Taylor J, Francois B *et al*. Lactic acidosis neurological deterioration and compromised cellular pyruvate oxidation due to a defect in the reoxidation of cytoplasmically generated NADH. *Eur J Pediatr* 1983; **140**: 98.
 61. Coude FX, Saudubray JM, Demangre F *et al*. Dichloroacetate as treatment for congenital lactic acidosis. *N Engl J Med* 1978; **299**: 1365.
 62. McKhann G, Francois B, Evrard P. Long term use of low doses of dichloroacetate in a child with congenital lactic acidosis. *Pediatr Res* 1980; **14**: 167.
 63. Spruijt L, Naviaux RX, McGowan KA *et al*. Nerve conduction changes in patients with mitochondrial diseases treated with dichloroacetate. *Muscle Nerve* 2001; **24**: 916.

Pyruvate carboxylase deficiency

Introduction	347	Treatment	352
Clinical abnormalities	348	References	352
Genetics and pathogenesis	351		

MAJOR PHENOTYPIC EXPRESSION

There are three phenotypes in each of which concentrations of lactic acid and alanine are elevated and activity of pyruvate carboxylase is deficient.

1. In the simple type common in American-Indians: delayed development and infantile episodes of metabolic acidosis with lactic acidemia.
2. In the complex type first described from France: severe lactic acidemia, usually fatal in the early months of life, hyperammonemia, citrullinemia and hyperlysinemia.
3. In a more benign presentation, episodic acidosis only.

INTRODUCTION

Pyruvate carboxylase (EC 6.4.1.1) is a biotin-containing mitochondrial enzyme, which catalyzes the conversion of pyruvate to oxaloacetate by CO_2 fixation (Figure 47.1) [1, 2]. As in the case of other carboxylases, the reaction mechanism is a two-step process in which biotin is first carboxylated and then the carboxyl group is transferred to the acceptor, pyruvate [3, 4]. There is a separate catalytic site for each of the two steps. The enzyme is a tetramer of 500 kDa whose individual equal-sized protomers have a different structure from other biotin-containing carboxylases [5], but the highly conserved amino acid sequence at the biotin

site of biotin-containing carboxylases, Ala-Met-Lys-Met is present in pyruvate carboxylase [6]. The biotin is linked to the ϵ amino group of the lysine.

Pyruvate carboxylase is an important regulatory enzyme with highly tissue-specific roles. In liver and kidney, where its activity is highest, it catalyzes the first step in gluconeogenesis from pyruvate in which the oxaloacetate formed is converted via phosphoenolpyruvate carboxykinase (PEPCK) to phosphoenolpyruvate, and ultimately to glucose and glycogen. It is regulated via acetylCoA, an allosteric activator, and the stimulant ratio of adenosine triphosphate/adenosine diphosphate (ATP/ADP). Conditions under which acetyl groups are

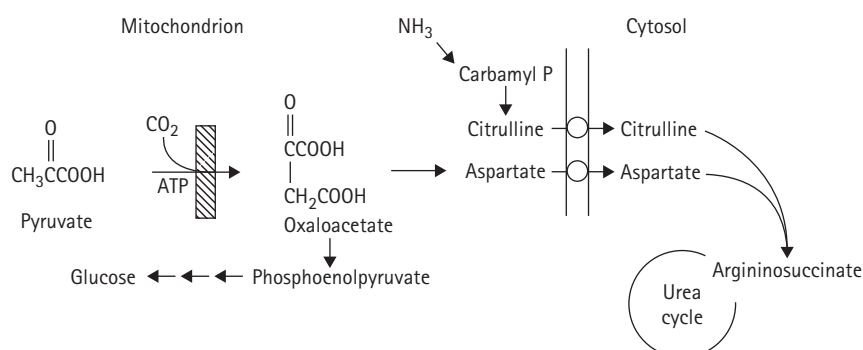


Figure 47.1 The pyruvate carboxylase reaction.



Figure 47.2 JM: A five-month-old infant with pyruvate carboxylase deficiency presented first in coma. He was hypotonic to flaccid and had inverted nipples. Respirations were Kussmaul. Metabolic imbalance was judged incompatible with life, but liver transplantation reversed many features of the disease.

generated stimulate gluconeogenesis at this step [7]. In lipogenic tissues, such as adipose and adrenal, the enzyme participates in the synthesis of acetyl groups and reducing groups for transport into the cytosol. In other tissues, such as brain, muscle, and fibroblasts, it has an anapleurotic role in the formation of oxaloacetate and the maintenance of four carbon intermediates for the citric acid cycle [8]. Anapleurotic is from the Greek verb to fill up. Experience with liver transplantation in this disease (Figure 47.2) [9] indicates that the liver can take over this citric acid cycle-related function for the entire body. In brain, the enzyme is active not in neurons but in astrocytes, where it is involved in the synthesis and supply for the neurons of glutamine, a major precursor of the glutamate and 4-aminobutyrate neurotransmitters [10].

Deficiency of pyruvate carboxylase was first described in patients with Leigh syndrome [11–14]. It is now thought that this was a function of instability of this enzyme, especially in material obtained at autopsy. In assays of biopsied liver in six patients with Leigh syndrome and of cultured fibroblasts in five, pyruvate carboxylase was not deficient [15].

The cDNA for pyruvate carboxylase has been cloned and sequenced and localized to chromosome 11 at q13.4–13.5 [6, 16–19]. Absence of mRNA for the enzyme was found in four of six patients with the fatal infantile disease who also lacked pyruvate carboxylase protein [20]. Mutations have been found in both the type A or simple form, as well as the type B severe infantile form [21], none of which patients had any enzyme activities. In a number of them, frame shifts led to premature terminations.

CLINICAL ABNORMALITIES

Complex, French, or European form

In the complex, French, or European form, severe neonatal lactic acidosis is the presenting picture [22, 23]. The initial acidosis may be fatal and many patients have died by three months of age. Most have hepatomegaly. Metabolic acidosis may lead to dehydration, coma, shock, and apnea. This disorder has now been observed in North American, Egyptian, and Saudi Arabian patients [9, 20, 24–26].

The term ‘complex’ refers to the biochemical findings in this group of patients in whom the occurrence of hyperammonemia and citrullinemia is characteristic [23, 25, 27]. Hyperlysinemia is also seen, as it is in other hyperammonemic conditions. Hypoglycemia may occur, but it is usually not a major problem. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) may be elevated. Concentrations of alanine and proline are high and there are elevated amounts of 2-oxoglutarate in the urine. Levels of lactate may be very high and levels of pyruvate are elevated. Abnormal redox balance in which the cytosol is more reducing is indicated by a high ratio of lactate to pyruvate in the blood, while a more oxidizing mitochondrial environment is indicated by a high acetoacetate to 3-hydroxybutyrate ratio [9]. The major metabolic abnormality, significant of citric acid cycle aberration, is the massive ketoacidosis. Ketoneuria is prominent.

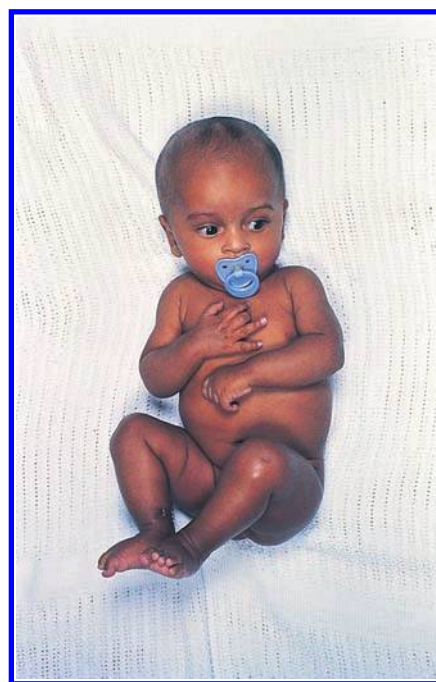


Figure 47.3 TYAAH: A six-month-old Saudi male infant with pyruvate carboxylase deficiency. He had recurrent episodes of lactic acidosis. Plasma ammonia was normal. Levels of alanine were elevated and proline was moderately elevated. Pyruvate carboxylase in fibroblasts was 11 percent of control.



Figure 47.4 TYAAH: The head was relatively large, just above 2 SD above the mean, while length and weight were below the 50th percentile. An electroencephalogram (EEG) showed diffuse slowing consistent with metabolic encephalopathy, and later he developed seizures. Computed tomography scans revealed a subdural effusion. By two years of age, he had spasticity and appreciably developmentally delayed.



Figure 47.6 OH: A previous sibling had died of intractable lactic acidosis.



Figure 47.5 OH: A six-month-old female infant with pyruvate carboxylase deficiency. She presented at 2 days of age with grand mal seizures. The tonic neck posture is normal at this age, but she also had choreoathetosis. She had lactic acidosis and undetectable activity of pyruvate carboxylase.



Figure 47.7 MSG: A 16-month-old Saudi male with pyruvate carboxylase deficiency. Tachypnea and acidosis were noted soon after birth. Initial pH was 7.00 and the serum bicarbonate was 5 mEq/L. Lactate was 6.2 mmol/L and alanine 1074 mmol/L; proline was 775 mmol/L. The activity of pyruvate carboxylase in fibroblasts was 4 percent of the control mean.

Simple or American-Indian form

In the simple or American-Indian form, there may be episodes of acute metabolic acidosis with lactic acidemia in the first six months of life, or the first evidence of abnormality may be slowness of development [28–30]. By the first year, most have impaired mental development and many have

failure to thrive, vomiting, or irritability. This clinical picture has been seen frequently in Saudi Arabia (Figures 47.3, 47.4, 47.5, 47.6, 47.7, and 47.8), as well as among American Indians [29]. It has been encountered in North



Figure 47.8 MSG: He had micrognathia and hypospadias. By 16 weeks, he could not sit unassisted or roll over. At 12 months, his head circumference was at the 50th percentile for four months, but length was at the same level and weight even further behind. Computed tomography scan revealed hypodense periventricular white matter.

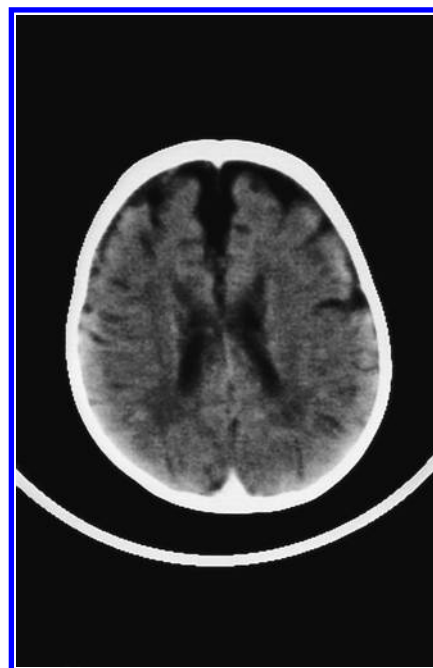


Figure 47.9 Computed tomography scan of the brain of the five-month-old patient shown in Figures 47.5 and 47.6. There was already marked atrophy of the brain, particularly evident over the frontal lobes. She died shortly after discharge.

American Caucasians and in Japanese [31]. Macrocephaly has been observed, as well as subdural effusions (Figures 47.4 and 47.5). These effusions have also been encountered in the complex phenotype [9]. Impaired mental development may be severe (Figures 47.5, 47.6, 47.7, and 47.8) [28]. Hypoglycemia occurred on fasting for 24 hours at which time the concentration of glucose was 1.8 mmol/L and lactate was 6.2 mmol/L, while the 3-hydroxybutyrate was greater than 2 mmol/L. A number of these patients have been ketotic at times of acute acidosis. There was no increase in blood glucose after an alanine load.

Seizures are frequently seen (Figure 47.5). One patient [28] presented at three months with fever and mild generalized seizures. Between the ages of five and nine months, he was admitted to hospital three times for failure to thrive and developmental delay; on each occasion, mild metabolic acidosis was noted, but not evaluated further. At nine months, he had a severe metabolic acidosis and was diagnosed as having lactic acidemia. The level was 6.5 mmol/L. By 46 months, he was microencephalic and had severely impaired mental development, unable to sit or feed himself, and not interested in his surroundings. He had numerous sudden episodes of lactic acidosis with tachypnea and a blotchy cyanosis of the extremities. He died at that age of pneumonia and severe acidosis. This patient and others in this group had proximal renal tubular acidosis. Renal tubular acidosis has also been encountered in the complex phenotype [9].

Electroencephalography (EEG) may show prominent theta waves and abnormal slow wave activity. Histologic examination of the brain has shown depletion of neurons and poor myelin formation, as well as increased ventricular size. These changes are evident in neuroimaging (Figure 47.9). Similar findings are seen in the complex form, in which there may also be cavitated infarcts or cortical cysts [22, 26]. Histologic examination of the liver in the simple and complex forms reveals steatosis.

Third form of pyruvate carboxylase deficiency

The third form of pyruvate carboxylase deficiency was described in a single patient [32], who had frequent episodic lactic acidosis in infancy. She was otherwise well and developed normally. By seven years of age, she had slight dysarthria and learning problems in mathematics. There was no failure to thrive; she was over the 95th percentile for height and weight.

Another patient reported as atypical [33], largely because of long survival, presented at 3 days of life with ketoacidosis, tachypnea, and hypotonia. By nine years, he had mild global developmental delay. Another unusual feature was magnetic resonance imaging (MRI) evidence of high signal intensities in the subcortical white matter of the frontal temporal area of the brain.

Molecular evidence of mutational heterogeneity may

ultimately recognize the three phenotypic distinctions as impractical. The metabolic abnormalities found in the complex phenotype are distinctive.

GENETICS AND PATHOGENESIS

All of the forms of pyruvate carboxylase deficiency appear to be inherited in an autosomal recessive fashion. A founder effect has been postulated for the abnormal gene in the Canadian Indians, all of whom speak the Algonquin language [34].

Enzyme activity can be measured in lymphocytes and cultured fibroblasts [20, 30], as well as tissues. Levels of enzyme activity have been very low, less than 5 percent of control, regardless of phenotype, but some activity is often measurable even in the most severely affected patients [9]. Assessment of the presence of pyruvate carboxylase enzyme protein has revealed differences. The enzyme can be labeled with ^3H -biotin or ^{35}S -streptavidin prior to sodium dodecyl sulfate (SDS) gel electrophoresis, which reveals a normal 125 kDa band in the two milder groups of patients, while no band was detected in the fatal severe neonatal form [20, 31]. Immunoprecipitation of ^{35}S -methionine-labeled protein with antibody to normal enzyme indicated absence of the protein in a number, but not all, of the patients with this form of the disease. Absence of mRNA for the enzyme was found in four of six patients with this form of the disease when tested by Northern blot assay with a cloned cDNA probe [20].

The cDNA for pyruvate carboxylase codes for 19 exons over 16 kb [35]. In studies of the rat gene, alternative tissue-specific transcripts led to greater expression in liver and kidney than in other tissues [36]. Among the Amerindian patients, homozygosity for G1828A which changed alanine 610 to threonine, was found in the Ojibwa or Cree, consistent with a founder effect. Carrier rates were as high as one in 10. In another group of Amerindians, there was a C2229T change, converting methionine 743 to isoleucine. In five unrelated patients with the severe infantile phenotype, there was at least one truncating mutation [21]. In two male siblings with this phenotype, there was compound heterozygosity for two deletions that were predicted to lead to frame shift, premature termination, and nonsense-mediated mRNA decay [37].

Heterozygote detection is sometimes possible in a family, but the range of normal is so great that a normal result may be inaccurate [36, 38]. At the other extreme, an apparent homozygote for the third type of disease with severe chronic lactic acidosis and no other abnormalities displayed 50 percent of control activity [39, 40].

Prenatal diagnosis has been accomplished in families at risk [41–43]. In one family in which a sibling had died of severe neonatal disease, biotin-labeled enzyme protein was absent in amniocytes.

The pathogenesis of lactic acidemia appears intuitively to be a direct consequence of the failure to metabolize

pyruvate by this pathway. Pyruvate does not accumulate, but is rather converted to alanine and lactate. Hypoglycemia has been observed [36] in each of the forms of the disease, and appears likely to be a consequence of a failure of gluconeogenesis.

The complex biochemical picture reminiscent of a defect in the urea cycle appears to result from depletion of intracellular oxaloacetate and aspartate [22, 23, 27]. Aspartate is a source of the second nitrogen of urea (Figure 47.1); its deficiency would lead to citrullinemia and hyperammonemia. Aspartate is also involved in the shuttle of reducing equivalents from cytosol to mitochondria [44] by which the NAD^+/NADH ratio (nicotinamide adenine dinucleotide/reduced nicotinamide adenine dinucleotide) is very oxidized in the cytosol and reduced in mitochondria; its lack would make the cytosol more reduced and the mitochondria more oxidized, as occurs in this phenotype.

Abnormality in the anapleurotic formation of glutamine in astrocytes is consistent with diminished concentrations of glutamine found in autopsied brain of a three-year-old patient [45]. Glutamine concentrations were low in the plasma and cerebrospinal fluid (CSF) of five living patients. Lactic acid itself may be directly toxic to brain. Depletion of oxaloacetate and interruption of the citric acid cycle would be expected to affect adversely the energy metabolism of the brain.

Experience with liver transplantation [9] has permitted a dissection of pathogenetic features of the disease. Ketoacidosis could be ameliorated by large amounts of intravenous glucose, but enteral glucose was much less effective and large amounts of enteral glucose markedly worsened the lactic acidosis. Orthotopic transplantation of the liver completely abolished life-threatening ketoacidosis and with it the systemic metabolic acidosis. Thus, it was clear that the acidosis and its enormous requirement for sodium bicarbonate to maintain neutrality is caused by the ketoacidosis, not by the lactic acidemia, because lactic acidemia, cerebral lactic acid elevation and lactic aciduria persisted.

The provision of enzyme in the transplanted liver also abolished the abnormal redox state of this disease in which the cytosol is reducing with a high lactate to pyruvate ratio, while the mitochondrial environment is more oxidizing as indicated by a high ratio of acetoacetate to 3-hydroxybutyrate.

Glutamine levels were low and did not improve with liver transplantation. This could reflect a role for glutamine depletion in the cerebral manifestations of this disease. Cerebral depletion of glutamine could affect the replenishment of glutamate and 4-aminobutyrate (GABA) neurotransmitter pools. Liver transplantation ameliorated the lactic acidemia, but lactic acid concentration in the cerebrospinal fluid remained elevated post-transplantation, and the lactate to pyruvate ratio was unchanged. The central nervous system effects of the disease were not reversed, but there was surprising improvement of function during the first year of follow-up evaluation.

TREATMENT

Metabolic acidosis and renal tubular acidosis have been treated in most patients with sodium bicarbonate. In acute episodes, parenteral fluids are required. A trial of biotin would appear prudent in any patient, but to date no responses have been reported.

Supplementation with aspartic acid appears to be a rational approach to a shortage of oxalacetate [9, 28, 46]. Treatment appeared to reduce levels of lactate and alanine and the number of acidotic attacks [28], but in our patient, much larger amounts of Na aspartate, along with Na citrate and Na succinate, failed to alter the life-threatening ketoacidosis. Glutamine 400–800 mg every 4 hours was thought to have diminished the number of acidotic episodes in a patient [28], but the disease proved relentless. Treatment with dichloroacetic acid is effective in ameliorating the lactic acidemia [9].

Hepatic transplantation abolished the renal tubular acidosis, as well as the ketoacidosis. None of the treatments reported have had a major effect on the cerebral features of the disease.

REFERENCES

- Utter MF, Barden RE, Taylor BL. Pyruvate carboxylase: an evaluation of the relationships between structure and mechanism and between structure and catalytic activity. *Adv Enzymol* 1975; **42**: 1.
- Scrutton MC, Young MR. Pyruvate carboxylase. In: Boyer PD (ed.). *The Enzymes*, vol. 6. New York: Academic Press, 1972: 1.
- McClure WR, Lardy HA, Wagner M, Cleland WW. Rat liver pyruvate carboxylase. II. Kinetic studies of the forward reaction. *J Biol Chem* 1971; **246**: 3579.
- McClure WR, Lardy HA, Cleland WW. Rat liver pyruvate carboxylase. III. Isotopic exchange studies of the first partial reaction. *J Biol Chem* 1971; **246**: 3584.
- Barden RE, Taylor BL, Isohashi F *et al*. Structural properties of pyruvate carboxylases from chicken liver and other sources. *Proc Natl Acad Sci USA* 1975; **72**: 4308.
- Freytag SW, Collier KJ. Molecular cloning of a cDNA for human pyruvate carboxylase. *J Biol Chem* 1984; **259**: 12831.
- Barrit GJ. Resolution of gluconeogenic flux by pyruvate carboxylase. In: Keech DB, Wallace JC (eds). *Pyruvate Carboxylase*. Boca Raton, FL: CRC Press, 1985: 141.
- Lee SH, Davis JE. Carboxylase and decarboxylation reactions anapleurotic flux and removal of citric acid cycle intermediates in skeletal muscle. *J Biol Chem* 1979; **254**: 420.
- Nyhan WL, Khanna A, Barshop BA *et al*. Pyruvate carboxylase deficiency: insights from liver transplantation. *Mol Genet Metab* 2002; **77**: 143.
- Shank RP, Bennett GS, Freytag SO, Campbell GL. Pyruvate carboxylase: an astrocyte-specific enzyme implicated in the replenishment of amino acid neurotransmitter pools. *Brain Res* 1985; **329**: 364.
- Hommes FA, Polman HA, Reerink JD. Leigh's encephalomyelopathy: an inborn error of gluconeogenesis. *Arch Dis Child* 1968; **43**: 423.
- Moosa A, Hughes EA. Proceedings: L-glutamine therapy in Leigh's encephalomyelopathy. *Arch Dis Child* 1974; **49**: 246.
- Van Biervliet JP, Duran M, Wadman SK *et al*. Leigh's disease with decreased activities of pyruvate carboxylase and pyruvate decarboxylase. *J Inherit Metab Dis* 1980; **2**: 15.
- Gilbert EF, Arya S, Chun R. Leigh's necrotizing encephalopathy with pyruvate carboxylase deficiency. *Arch Pathol Lab Med* 1983; **107**: 126.
- Murphy JV, Isohashi F, Weinberg MB, Utter MF. Pyruvate carboxylase deficiency: an alleged biochemical cause of Leigh's disease. *Pediatrics* 1981; **68**: 401.
- Lamhonwah A, Quan F, Gravel RA. Sequence homology around biotin-binding site of human propionyl-CoA carboxylase and pyruvate carboxylase. *Arch Biochem Biophys* 1987; **254**: 631.
- Lim F, Morris CP, Occhiodoro F, Wallace JC. Sequence and domain structure of yeast pyruvate carboxylase. *J Biol Chem* 1988; **263**: 11493.
- Zhang J, Xia W-L, Brew K, Ahmand F. Adipose pyruvate carboxylase: amino acid sequence and domain structure deduced from cDNA sequencing. *Proc Natl Acad Sci USA* 1993; **90**: 1766.
- Walker ME, Baker E, Wallace JC, Sutherland GR. Assignment of the human pyruvate carboxylase gene (PC) to 11q134 by fluorescence *in situ* hybridization. *Cytogenet Cell Genet* 1995; **69**: 187.
- Robinson BH, Oei J, Saudubray JM *et al*. The French and North American phenotypes of pyruvate carboxylase deficiency. Correlation with biotin containing protein by 3H-biotin incorporation 35S-streptavidin labeling and Northern blotting with a cloned cDNA probe. *Am J Hum Genet* 1987; **40**: 50.
- Monnot S, Serre V, Chadeaux-Vekemans B *et al*. Structural insights on pathogenic effects of novel mutations causing pyruvate carboxylase deficiency. *Hum Mutat* 2009; **30**: 734.
- Saudubray JM, Marsac C, Charpentier C *et al*. Neonatal congenital lactic acidosis with pyruvate carboxylase deficiency in two siblings. *Acta Paediatr Scand* 1976; **65**: 717.
- Coude FX, Ogier H, Marsac C *et al*. Secondary citrullinemia with hyperammonemia in four neonatal cases of pyruvate carboxylase deficiency. *Pediatrics* 1981; **68**: 914.
- Bartlett K, Ghneim HK, Stirk JH *et al*. Pyruvate carboxylase deficiency. *J Inherit Metab Dis* 1984; **7**: 74.
- Greter J, Gustafsson J, Holme E. Pyruvate carboxylase deficiency with urea cycle impairment. *Acta Paediatr Scand* 1985; **74**: 982.
- Wong LTK, Davidson GF, Applegarth DA *et al*. Biochemical and histologic pathology in an infant with cross-reacting material (negative) pyruvate carboxylase deficiency. *Pediatr Res* 1986; **20**: 274.
- Charpentier C, Tetau JM, Ogier H *et al*. Amino acid profile in pyruvate carboxylase deficiency: comparison with some other metabolic disorders. *J Inherit Metab Dis* 1982; **5**(Suppl. 1): 11.
- Atkin BM, Buist NR, Utter MF *et al*. Pyruvate carboxylase deficiency and lactic acidosis in a retarded child without Leigh's disease. *Pediatr Res* 1979; **13**: 109.

29. Haworth JC, Robinson BH, Perry TL. Lactic acidosis due to pyruvate carboxylase deficiency. *J Inherit Metab Dis* 1981; **4**: 57.
30. DeVivo DC, Haymond MW, Leckie MP *et al*. The clinical and biochemical implications of pyruvate carboxylase deficiency. *J Clin Endocrinol Metab* 1977; **45**: 1281.
31. Robinson BH, Oei J, Sherwood WG *et al*. The molecular basis for the two different clinical presentations of classical pyruvate carboxylase deficiency. *Am J Hum Genet* 1984; **36**: 283.
32. Van Coster RN, Fernhoff PM, DeVivo DC. Pyruvate carboxylase deficiency: a benign variant with normal development. *Pediatr Res* 1991; **30**: 1.
33. Schiff M, Levrat V, Acquaviva C *et al*. A case of pyruvate carboxylase deficiency with atypical clinic and neuroradiological presentations. *Mol Genet Metab* 2006; **87**: 175. Epub 2005 Dec 1.
34. Robinson BH. Lactic acidemia: biochemical clinical and genetic considerations. In: Harris H, Hirschborn K (eds). *Advances in Human Genetics*. New York: Plenum Press, 1989: 151.
35. Carbone MA, MacKay N, Ling M *et al*. Amerindian pyruvate carboxylase deficiency is associated with two distinct missense mutations. *Am J Hum Genet* 1998; **62**: 1312.
36. Jitrapakdee S, Booker GW, Cassady AI, Wallace JC. The rat pyruvate carboxylase gene structure. Alternate promoters generate multiple transcripts with the 5-end heterogeneity. *J Biol Chem* 1997; **272**: 20522.
37. Carbone MA, Applegarth DA, Robinson BH. Intron retention and frameshift mutations result in severe pyruvate carboxylase deficiency in two male siblings. *Hum Mutat* 2002; **20**: 48.
38. Gravel RA, Robinson BH. Biotin-dependent carboxylase deficiencies (propionyl-CoA and pyruvate carboxylase). *Ann NY Acad Sci* 1985; **447**: 225.
39. Hansen TL, Christensen E, Willems JL, Trijbels JMF. A mutation of pyruvate carboxylase in fibroblasts from a patient with severe chronic lactic acidemia. *Clin Chim Acta* 1983; **131**: 39.
40. Brunette MG, Delvin E, Hazel B, Scriver CR. Thiamine-responsive lactic acidosis in a patient with deficient low Km pyruvate carboxylase activity in liver. *Pediatrics* 1972; **50**: 702.
41. Marsac C, Augerau GL, Feldman G *et al*. Prenatal diagnosis of pyruvate carboxylase deficiency. *Clin Chim Acta* 1982; **119**: 121.
42. Robinson BH, Toon JR, Petrova-Benedict R *et al*. Prenatal diagnosis of pyruvate carboxylase deficiency. *Prenat Diagn* 1985; **5**: 67.
43. Tsuchiyama A, Oyanagi K, Hirano S *et al*. A case of pyruvate carboxylase deficiency with later prenatal diagnosis of an unaffected sibling. *J Inherit Metab Dis* 1983; **6**: 85.
44. Robinson BH, Halperin ML. Transport of reduced nicotinamide adenine dinucleotide into mitochondria of white adipose tissue. *Biochem J* 1985; **116**: 229.
45. Perry TL, Haworth JC, Robinson BH. Brain amino acid abnormalities in pyruvate carboxylase deficiency. *J Inherit Metab Dis* 1985; **8**: 63.
46. Ahmad A, Kahler SG, Kishnani PS *et al*. Treatment of pyruvate carboxylase deficiency with high doses of citrate and aspartate. *Am J Med Genet* 1999; **87**: 331.

Fructose-1,6-diphosphatase deficiency

Introduction	354	Treatment	357
Clinical abnormalities	355	References	357
Genetics and pathogenesis	356		

MAJOR PHENOTYPIC EXPRESSION

Hypoglycemia, lactic acidosis-impaired gluconeogenesis, and deficiency of hepatic fructose-1,6-diphosphatase.

INTRODUCTION

Deficiency of fructose-1,6-diphosphatase (FDP) (fructose-1,6-bisphosphatase) was first recognized in 1970 by Baker and Winegrad [1], in a girl with hypoglycemia and metabolic acidosis. A sibling had died of a similar illness. In subsequent reports in 1971 by Baerlocher and colleagues [2] and by Hulsmann and Fernandez [3], there were multiple affected siblings of consanguineous matings.

The enzyme FDP (EC 3.1.3.11) provides an essential step in the pathway of gluconeogenesis (Figure 48.1). The

enzyme catalyzes the irreversible conversion of fructose-1,6-diphosphate to fructose-6-phosphate. Another enzyme, phosphofructokinase, and adenosine triphosphate (ATP) are required to take this reaction in the reverse direction. The enzyme is most active in liver and kidney; and the liver enzyme is highly regulated [4]. Deficiency has most often been documented in biopsied liver. The gene (*FBP1*) has been cloned and localized to chromosome 9q22.2-22.3 [5]. Seven exons span 31 kb. The common mutation in Japanese people is an insertion, 960–961insG [6], which was also the most frequent mutation in a non-Japanese population

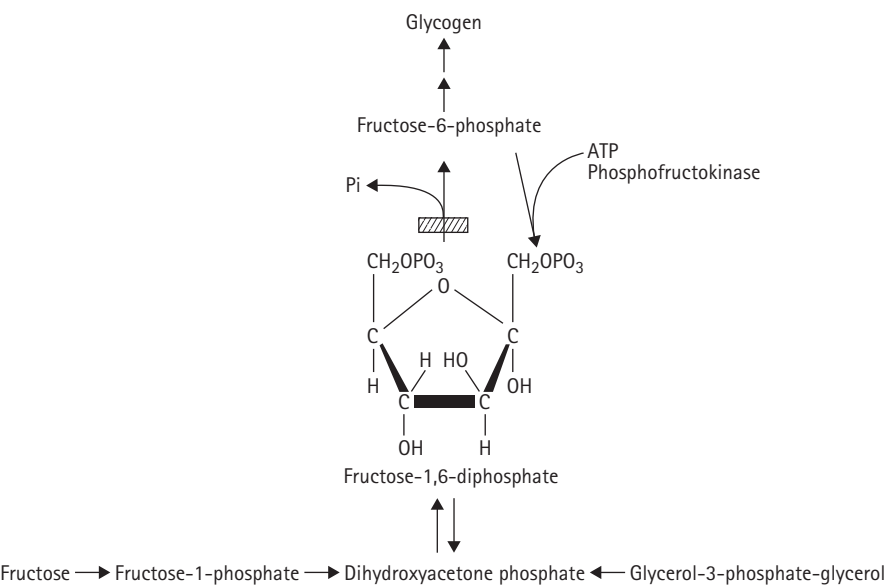


Figure 48.1 The fructose-1,6-diphosphatase reaction and its role in gluconeogenesis. The conversion of fructose-6-phosphate to fructose-1,6-diphosphate in glycolysis is catalyzed by another enzyme, 6-phosphofructose-1-kinase.

[7, 8]. This mutation causes a frame shift and premature chain termination, as does 966del, and expression studies have shown both to be pathogenic. The disease is clearly genetically heterogeneous and a variety of other mutations has been found.

CLINICAL ABNORMALITIES

FDP deficiency is a cause of life-threatening metabolic acidosis in the neonatal period. A history of a previous sibling who died in acidosis has often been the alerting episode that led to early diagnosis and survival in the subsequent affected infant [1, 2]. Onset in about 50 percent of patients is between 1 and 4 days of age. Most of the patients present before six months. An exception first developed symptoms at four years [9].

The first symptom in the neonatal presentation is usually hyperventilation. There may be irritability, but progression is usually rapid to somnolence, coma, apnea, and cardiac arrest [2]. Physical examination may reveal tachycardia and hepatomegaly. Laboratory evaluation reveals hypoglycemia, severe acidosis, and lactic acidemia [10]. The episode usually responds well to vigorous therapy with parenteral fluids containing glucose and sodium bicarbonate (Figures 48.2 and 48.3).

Subsequent episodes usually follow fasting, usually precipitated by intercurrent infections. Onset may be



Figure 48.2 NM: An infant with fructose-1,6-diphosphatase deficiency. She did not have neonatal hypoglycemia. Her first episodes occurred following exposure to fruit juices in the infant's diet.



Figure 48.3 NM: Close up of the face, an appearance consistent with her normal intelligence.

with vomiting and anorexia; attendant fasting leads to hypoglycemia and metabolic acidosis. In one patient, episodes of hyperventilation began when the infant was weaned and baby foods were begun at six months [10]; on admission, she was hypoglycemic and the lactic acid concentration was 20 mmol/L. Patients have been described as ketotic and the urinary test for ketones is often positive during the acute episode, but the disease has been classified among hypoketotic causes of metabolic acidosis and coma [11]. In the absence of gluconeogenesis, ketones would be expected to accumulate as soon as hepatic glycogen is depleted, and the usual crisis is associated with ketosis. Vomiting is not a common response to fructose and patients do not have the aversion to fruit and its products, seen regularly in hereditary fructose intolerance. Nor do they develop proximal renal tubular dysfunction after fructose, as do those former patients.

Hepatomegaly develops regularly in infancy, but there are usually no signs of liver disease [11, 12]. Neonatal hyperbilirubinemia of a severity requiring exchange transfusion was reported in three infants [13]. Failure to thrive may be seen rarely.

There may be convulsions or other manifestations of hypoglycemia. There may be flushing [2], or pallor and sweating. Vomiting may be complicated by hematemesis [14, 15]. Hypotonia and muscle weakness have been observed. The electroencephalograph (EEG) may be abnormal during the acute attack and normal later. Fast spindle-shaped bursts on a slow amplitude pattern have been described [2], as well as a slow wave pattern [16]. Intellectual development is usually normal (Figures 48.2

and 48.3). Of course, impaired mental development, as well as death may accompany neonatal or early infantile hypoglycemic crises, but fasting tolerance improves with age, and patients normal by childhood usually develop normally. In addition to the lactic acidemia, analysis of the blood reveals increased concentrations of alanine and uric acid [17]. In some attacks, there may be acidosis without hypoglycemia. Glycerol and glycerol-3-phosphate have been found in the urine [18, 19].

GENETICS AND PATHOGENESIS

The disease is transmitted in an autosomal recessive fashion. There is no ethnic predominance. Consanguinity was observed early [2, 3]. Most reports have been of Europeans, but there have been reports from the United States, Japan, and Lebanon [10, 20–25], and among the European families in one series a majority were Turkish [25]. Among nine patients from six families in Israel, two families were Jewish, three Arabic, and one Druze [26]. The disease is common in Saudi Arabia, where 14 patients were reported [27]. Parents of patients have been documented to have intermediate levels of FDP activity in liver [14, 28]. Testing for heterozygosity by assay of the enzyme in leukocytes may be unreliable. Prenatal diagnosis and heterozygote detection can be accomplished if the mutation is known.

The defective enzyme in patients has usually been identified in the assay of biopsied liver [1–3, 29]; a majority of patients have little or no activity. Others have had 20 percent or less of control activity. The enzyme is also active in kidney [3, 15] and jejunum [30], and the defect has been identified in both these tissues. Assay of leukocytes is controversial and the activity in normal individuals is quite low. The enzyme is not expressed in fibroblasts or amniocytes. The defect has been documented in tissues obtained at autopsy, but the results must be interpreted with caution because of rapid inactivation of the enzyme with autolysis [31]. The documentation of FDP enzyme activity in muscle in patients in whom activity was deficient in liver and kidney indicates that the enzyme in muscle is coded by a different gene [10, 15]. Diagnosis has generally required the assay of the enzyme in biopsied liver, but mutational analysis may permit its avoidance. The enzyme in blood is expressed only in monocytes. Culture of monocytes in media rich in calcitriol has been reported [32] to permit the diagnosis by enzyme assay. A radiochemical method has developed that detects defective (absent) enzyme activity in isolated monocytes before and after incubation with calcitriol in tissue culture media [33].

The gene *FBP1* on chromosome 9 has seven exons. The insG960-961 accounted for 46 percent of 22 mutant alleles in Japanese and 14 of 28 mutant alleles in non-Japanese [7]. Its frequency in diverse populations suggests a propensity for mutation. Frame shift was also observed with 807delG and 704-705insC [7]. Point mutations included A177D, N213K, and G294V [7, 32]. Premature termination

(Q30X) was homozygous in a Japanese patient [6]. In a small number of patients, a concerted search for mutation has failed to reveal any [6]; the possibility of mutation in a promoter region has been suggested. In a Japanese patient, C851G leading to p.F194S was found in compound with p.P284R [34]. In a Swedish patient, two heterozygous mutations were p.G620R and p.G294E, while another had p.Y216X and a large 300-kb deletion [33].

Deficient activity of FDP interferes with gluconeogenesis, making the patient dependent on exogenous sources of glucose. The fasting that normal infants often undergo in the first days of life makes for a neonatal presentation in patients with little residual enzyme activity [35]. If tested with a provocative fast, patients become hypoglycemic when stored glycogen is exhausted. This may occur in a few hours in an infant [15] or after 14–20 hours in an older patient [1, 2, 15, 34]. Depletion of glycogen previously synthesized from glucose by injection of glucagon early in the fast ensures that gluconeogenesis is being tested adequately (Chapter 46) [10]. The fed state response to glucagon may be normal. Administration of glucagon after the development of hypoglycemia leads to no glycemic response. In FDP deficiency, hypoglycemia induced by fasting is accompanied by increases in levels of lactate, pyruvate, and alanine [2, 15, 36], along with acetoacetate and 3-hydroxybutyrate. Hypoglycemia may also be generated by a diet low in carbohydrate and high in protein and fat [10].

Fructose loading yields evidence of fructose intolerance. This test has been employed in patients judged on the basis of the response to fasting to have lactic acidemia, due to a defect in gluconeogenesis (Chapter 46), in order to suggest that the enzyme be assayed on biopsied liver. It should, nevertheless, be undertaken with caution, as there is risk of severe reaction. An intravenous test is preferred unless hereditary fructose intolerance can be excluded, although patients with FDP deficiency do not develop intestinal symptoms after an oral load [1, 2]. The preferred intravenous dose is 200 mg/kg [31, 37]. The response is dose-related. A patient became comatose after 500 mg/kg i.v. [16]. Following fructose, the blood sugar drops to hypoglycemic levels within 15 minutes; lactic acidemia and increased levels of alanine and uric acid accompany systemic acidosis. Fructose administration may induce hyperuricemia and uricosuria even in normal individuals, and the accumulated uric acid results from degradation of adenine nucleotides, following the utilization of ATP in the fructokinase reaction; phosphorus depletion results from the rephosphorylation of adenosine diphosphate (ADP).

These patients are also intolerant of glycerol and sorbitol. Glycerol loading leads to a response similar to that with fructose [1, 7, 30, 38]. Concentrations of phosphate also fall. Sorbitol has been infused to treat cerebral edema; in a child not realized to have FDP deficiency and thought to have cerebral edema, repeated infusions of sorbitol were lethal [28]. Patients with FDP deficiency have normal tolerance of galactose.

Table 48.1 Carbohydrate content of common medications (modified from Bosso *et al.* [39])

Medication	Presentation	Sugar form	Manufacturer
ADC	Drops	Sucrose	Parke-Davis
Actifed	Syrup	Sucrose	Burroughs-Wellcome
Amcill 250	Suspension	Sucrose	Parke-Davis
Benadryl	Elixir	Sucrose	Parke-Davis
Betapen-VK	Solution	Glucose	Bristol
Cascara	Liquid (FE 536)	Sucrose	Parke-Davis
Sagrada aromatic Fluid extract			
Chlor-Trimeton	Syrup	Sucrose	Schering
Compazine	Syrup	Sucrose	Smith, Kline & French
Compocillin-VK drops	Drops	Sucrose	Abbott
Dilantin-30	Suspension	Sucrose	Smith, Kline & French
Erythrocin	Drops	Sucrose	Mead Johnson
Fer-in-sol	Syrup	Sucrose	Abbott
Gantrisin	Syrup	Sucrose	Roche
Ilosone 125	Liquid	Sucrose	Lilly
Keflex	Suspension	Sucrose	Lilly
Lomotil	Liquid	Sucrose	Searle
Phenobarbital	Elixir	Sucrose	Lilly
Polycillin	Suspension	Sucrose	Philips Roxane
Robicillin VK 125 solution	Solution	Sucrose	Robins
Robitussin	Syrup	Glucose and sucrose	Robins
Sudafed	Syrup	Sucrose	Burroughs-Wellcome
Tylenol	Elixir	Sucrose	McNeil

TREATMENT

Treatment of the acute episode of hypoglycemia and lactic acidosis is the prompt administration of generous amounts of fluid, sodium bicarbonate, and glucose. The episode usually responds readily. Avoidance of fasting is an important element of subsequent management and if the oral route is temporarily compromised by vomiting, or intercurrent illness, an intravenous supply of glucose is mandatory.

Dietary fructose and sucrose are avoided. In most patients, they do not have to be absolutely eliminated. Rather, the individual tolerance of the patient can be explored cautiously [14], while soft drinks that provide a sucrose or fructose load should be avoided. Lists are available [37] that provide the sugar content and its nature of medicinal liquids. An abbreviated list is shown in [Table 48.1](#). Patients, families, and physicians should particularly be warned about antibiotic elixirs or tylenol syrup which tend to be prescribed when the patient is already metabolically compromised with infection and vomiting-

related fasting. A snack at bedtime is often useful, as is uncooked cornstarch ([Chapters 38 and 59](#)).

With treatment, hepatomegaly recedes. Subsequent episodes can largely be avoided or aborted. Tolerance to fasting improves with age [1]. Long-term prognosis may be excellent [33]. One patient developed gout at 32 years of age and was treated with allopurinol [33].

REFERENCES

1. Baker L, Winegrad AI. Fasting hypoglycemia and metabolic acidosis associated with deficiency of hepatic fructose-1,6-diphosphatase activity. *Lancet* 1970; **2**: 13.
2. Baerlocher K, Gitzelmann R, Nussli R, Dumermuth G. Infantile lactic acidosis due to hereditary fructose-1,6-diphosphatase deficiency. *Helv Paediatr Acta* 1971; **26**: 489.
3. Hulsmann WC, Fernandez J. A child with lactic acidemia and fructose-1,6-diphosphatase deficiency in the liver. *Pediatr Res* 1971; **5**: 633.
4. Benkovic SJ, Demaine MM. Mechanism of action of fructose-1,6-bisphosphatase. *Adv Enzymol* 1982; **53**: 45.

5. El-Maghrabi MR, Lange AJ, Jiang W *et al.* Human fructose-1, 6-bisphosphatase gene (FBP1): exon-intron organization, localization to chromosome bands 9q22.2-q22.3, and mutation screening in subjects with fructose-1, 6-bisphosphatase deficiency. *Genomics* 1995; **27**: 520.
6. Kikawa Y, Inuzuka M, Jin BY *et al.* Identification of genetic mutations in Japanese patients with fructose-1,6-bisphosphatase deficiency. *Am J Hum Genet* 1997; **61**: 852.
7. Herzog B, Morris AAM, Saunders C, Eschrich K. Mutation spectrum in patients with fructose-1,6-bisphosphatase deficiency. *J Inherit Metab Dis* 2001; **24**: 87.
8. Herzog B, Wendel U, Morris AAM, Eschrich K. Novel mutations in patients with fructose-1,6-bisphosphatase deficiency. *J Inherit Metab Dis* 1999; **22**: 132.
9. De Pr  M, Laudanna E. La malattia di Baker-Winegard. *Minerva Pediatr* 1978; **30**: 1973.
10. Pagliara AS, Karl IE, Keating JP *et al.* Hepatic fructose-1,6-diphosphatase deficiency. A cause of lactic acidosis and hypoglycemia in infancy. *J Clin Invest* 1972; **51**: 2115.
11. Saudubray JM, Charpentier C. Clinical phenotypes: diagnosis/ algorithms. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic and Molecular Bases of Inherited Disease*. New York: McGraw Hill, 1995: 327.
12. Eagle RB, MacNab AJ, Ryman BE, Strang LB. Liver biopsy data on a child with fructose-1,6-diphosphatase deficiency that closely resembled many aspects of glucose-6-phosphatase deficiency (Von Gierke's type 1 glycogen-storage disease). *Biochem Soc Trans* 1974; **2**: 1118.
13. Buhrdel P, Bohme H-J, Didt L. Biochemical and clinical observations in four patients with fructose-1,6-diphosphatase deficiency. *Eur J Pediatr* 1990; **149**: 574.
14. Saudubray J-M, Dreyfus J-C, Capanec C *et al.* Acidose lactique hypoglyc  mie et h  patom  galie par d  ficit h  r  ditaire en fructose-1,6-diphosphatase h  patique. *Arch Fr P  diatr* 1973; **30**: 609.
15. Melancon SB, Khacaturian AK, Nadler HL, Brown BI. Metabolic and biochemical studies in fructose-1,6-diphosphatase deficiency. *J Pediatr* 1973; **82**: 650.
16. Corbeel L, Eggermont E, Eeckels R *et al.* Recurrent attacks of ketotic acidosis associated with fructo-1,6-diphosphatase deficiency. *Acta Paediatr Belg* 1976; **29**: 29.
17. Hopwood NJ, Holzman I, Drash AL. Fructose-1,6-diphosphatase deficiency. *Am J Dis Child* 1977; **131**: 418.
18. Dremsek PA, Sacher M, St  gmann W *et al.* Fructose-1,6-diphosphatase deficiency: glycerol excretion during fasting test. *Eur J Pediatr* 1985; **144**: 203.
19. Krywawych S, Katz G, Lawson AM *et al.* Glycerol-3-phosphate excretion in fructose-1,6-diphosphatase deficiency. *J Inherit Metab Dis* 1986; **9**: 388.
20. Kinugasa A, Kusunoki T, Iwashima A. Deficiency of glucose-6-phosphate dehydrogenase found in a case of hepatic fructose-1,6-diphosphatase deficiency. *Pediatr Res* 1979; **13**: 1361.
21. Ito M, Kuroda Y, Kobashi H *et al.* Detection of heterozygotes for fructose-1,6-diphosphatase deficiency by measuring fructose-1,6-diphosphatase activity in their cultured peripheral lymphocytes. *Clin Chim Acta* 1984; **141**: 27.
22. Nakai A, Shigematsu Y, Liu YY *et al.* Urinary sugar phosphates and related organic acids in fructose-1,6-diphosphatase deficiency. *J Inherit Metab Dis* 1993; **16**: 408.
23. Nagai T, Yokoyama T, Hasegawa T *et al.* Fructose and glucagon loading in siblings with fructose-1,6-diphosphatase deficiency in fed state. *J Inherit Metab Dis* 1992; **15**: 720.
24. Alexander D, Assaf M, Khudr A *et al.* Fructose-1,6-diphosphatase deficiency: diagnosis using leukocytes and detection of heterozygotes with radiochemical and spectrophotometric method. *J Inherit Metab Dis* 1985; **8**: 147.
25. Gitzelmann R, Baerlocher K, Prader A. Heredit  re St  rungen im Fructose- und Galaktosestoffwechsel. *Monatsschr Kinderheilkd* 1973; **121**: 174.
26. Moses SW, Bashan N, Flasterstein BF *et al.* Fructose-1,6-diphosphatase deficiency in Israel. *Isr Med J* 1991; **27**: 1.
27. Rashed M, Ozand PT, Al Aqeel A, Gascon GG. Experience of King Faisal Specialist Hospital and Research Center with Saudi organic acid disorders. *Brain Dev* 1994; **16**(Suppl.): 1.
28. Baerlocher K, Gitzelmann R, Steinmann B. Clinical and genetic studies of disorders in fructose metabolism. In: Burman D, Holton JB, Pennock CA (eds). *Inherited Disorders of Carbohydrate Metabolism*. Lancaster: MTP, 1980: 163.
29. Gitzelmann R. Enzymes of fructose and galactose metabolism: galactose-1-phosphate. In: Curtius H-C, Roth M (eds). *Clinical Biochemistry: Principles and Methods*. Berlin: Gruyter, 1974: 1236.
30. Greene HL, Stifel FB, Herman RH. 'Ketotic hypoglycemia' due to hepatic fructose-1,6- diphosphatase deficiency. *Am J Dis Child* 1972; **124**: 415.
31. Steinmann B, Gitzelmann R. The diagnosis of hereditary fructose intolerance. *Helv Paediatr Acta* 1981; **36**: 297.
32. Kikawa Y, Shin YS, Inuzuka M *et al.* Diagnosis of fructose-1,6-bisphosphatase deficiency using cultured lymphocyte fraction: a secure and noninvasive alternative to liver biopsy. *J Inherit Metab Dis* 2002; **25**: 41.
33. Asberg C, Hjalmarson O, Alm J *et al.* Fructose 1, 6-bisphosphatase deficiency: enzyme and mutation analysis performed on calcitriol-stimulated monocytes with a note on long-term prognosis. *J Inherit Metab Dis* 2010; DOI:10.1007/s10545-009-9034-5.
34. Matsuura T, Chinen Y, Arashiro R *et al.* Two newly identified genomic mutations in a Japanese female patient with fructose-1,6-bisphosphatase (FBPase) deficiency. *Mol Genet Metab* 2002; **76**: 207.
35. Pagliara AS, Karl IE, Hammond M, Kipnis DM. Hypoglycemia in infancy and childhood. Parts I and II. *J Pediatr* 1973; **82**: 365.
36. Rallison ML, Meikle AW, Zigrang WD. Hypoglycemia and lactic acidosis associated with fructose-1,6-diphosphatase deficiency. *J Pediatr* 1979; **94**: 933.
37. Steinmann B, Gitzelmann R. Fruktose und Sorbitol in Infusionsfl  ssigkeiten sind nicht immer harmlos. *Int J Vitam Nutr Res Suppl* 1976; **15**: 289.
38. Odi  vre M, Brivet M, Moatti N *et al.* D  ficit en fructose-1,6-diphosphatase chez deux soeurs. *Arch Fr P  diatr* 1975; **32**: 113.
39. Bosso JA, Pearson RE. Sugar content of selected liquid medicinals. *Diabetes* 1973; **22**: 776.

Deficiency of the pyruvate dehydrogenase complex

Introduction	359	Treatment	364
Clinical abnormalities	360	References	364
Genetics and pathogenesis	362		

MAJOR PHENOTYPIC EXPRESSION

Acute, potentially lethal metabolic acidosis, hyperventilation, Leigh syndrome, hypotonia, ataxia, failure to thrive or developmental impairment, elevated concentrations of lactic and pyruvic acids and alanine in blood, urine, and cerebrospinal fluid; and defective activity of the pyruvate dehydrogenase complex.

INTRODUCTION

The pyruvate dehydrogenase complex (PDHC) is a mitochondrial multienzyme system that catalyzes the oxidation of pyruvate to CO₂ and acetylCoA and concomitantly generates reduced nicotinamideadenine-dinucleotide (NADH) (Figure 49.1) [1]. Cofactors include thiaminepyrophosphate (TPP), lipoic acid, coenzyme A (CoA), flavineadeninedinucleotide (FAD), and nicotinamideadeninedinucleotide (NAD1); Mg is required. There are eight different protein components, in seven of which human deficiency disease has been documented. The three basic components (E1, E2, and E3) are functional catalytic proteins, of types that are shared by all oxoacid dehydrogenases.

The reaction catalyzed by E1, the first enzyme in the complex (EC 1.2.4.1), which has been referred to as pyruvate decarboxylase (PDC) and contains TPP, accomplishes the oxidative decarboxylation of pyruvate to CO₂ and the linkage of the remaining two-carbon unit to TPP to form a hydroxyethylthiamine pyrophosphate attached to the enzyme (TTP-E1).

The second enzyme, E2 (EC 2.3.1.12), dihydrolipoyl transacetylase, is an acyl transferase; it catalyzes the transfer of the hydroxyethyl group and its oxidation to acetylCoA (Figure 49.1). Concomitantly, the disulfide bridge of the lipoic acid moiety attached to E2 is reduced to the SH form. This attached dihydrolipoic acid is reoxidized in the reaction catalyzed by the E3 enzyme, dihydrolipoyl dehydrogenase or lipoamide dehydrogenase (EC 1.6.4.3). The same E3

component is shared by 2-oxoglutarate dehydrogenase and the branched-chain ketoacid decarboxylase, providing a mechanism for patients who have defective activity in all three systems (Chapter 50) [2]. In PDHC, a lipoyl-containing catalytic protein has been referred to as protein X, which also functions in acyl transfer [3, 4]. Lipoic acid is attached to the E2 or X protein covalently to lysine moieties. Protein X may also serve in binding E3 to the rest of the complex [5].

Regulation of PDHC involves covalent modification of the protein to produce active and inactive forms. The active form is the dephosphorylated one, and this reaction is catalyzed by a specific phosphatase (PDH phosphatase). A few patients, including an infant with fatal infantile lactic acidemia, have been described in whom the inactive enzyme could not be dephosphorylated because PDH phosphatase activity was deficient [6]. Phosphorylation is catalyzed by a specific kinase, PDH kinase. Additional regulation of PDHC is via end-product inhibition by NADH and acetylCoA [1, 7]. Insulin also activates the enzyme, by fostering the prevalence of the dephosphorylated form. Dichloroacetic acid (DCA) inhibits the kinase, thus keeping the gate to the citric acid cycle locked in the open position [8].

The E1 enzyme is a tetramer of α and β subunits in an $\alpha_2\beta_2$ form. The α protein is 41 kDa and the β is 36 kDa. It is the E1 α subunit that is phosphorylated and dephosphorylated by the kinase and phosphatase at serine residues [9].

Lactic acid is the major end product of anaerobic

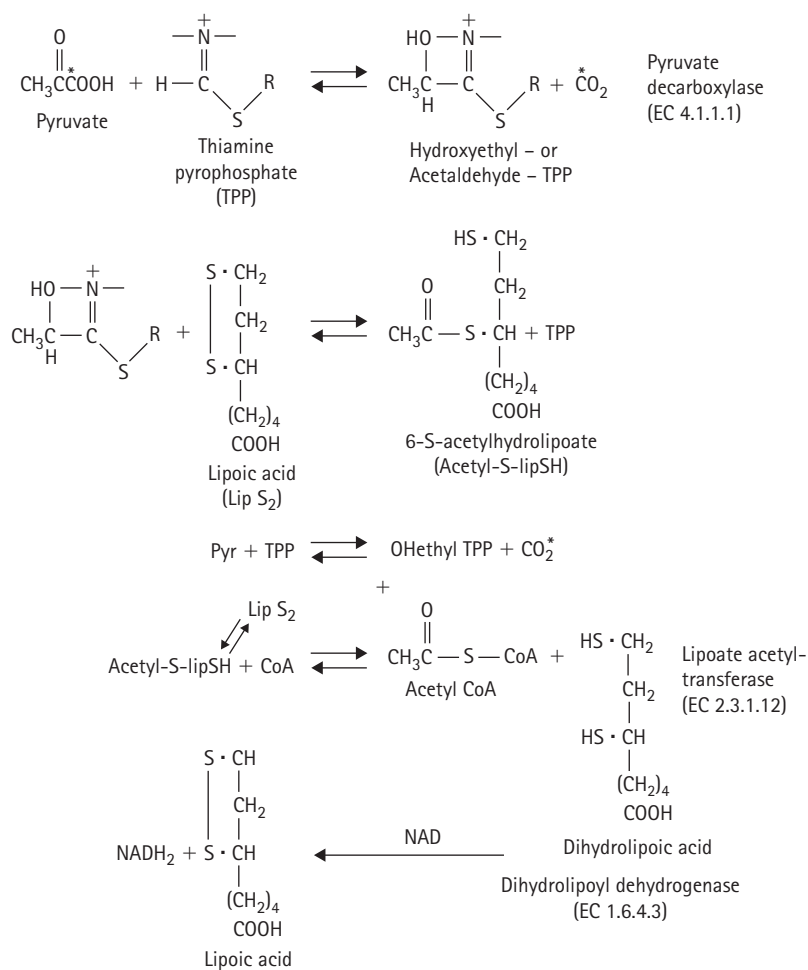


Figure 49.1 The pyruvate dehydrogenase complex (PDHC).

glycolysis. It accumulates whenever production of pyruvic acid exceeds utilization. This occurs temporarily during exercise in which there is an oxygen debt, as well as in conditions in which oxygen supplies decrease because of cardiac or respiratory insufficiency or vascular perfusion problems, such as shock. There is also cyclic interconversion of lactate in the Cori cycle in which glucose is converted to lactate in peripheral tissues such as brain and muscle, which oxidize carbohydrates largely to CO_2 and water, and the lactate formed circulates to the liver, where it is converted to glucose [10]. Because of gluconeogenesis, the amount of carbohydrate supplied to peripheral tissues from the liver exceeds the amount ingested. The brain is a major site of its oxidation, and this percentage is greater in infancy and childhood because of the greater proportion of the brain size to body size. The dependency of the brain on oxidative metabolism makes it particularly susceptible to damage in diseases of oxidation that lead to lactic acidosis. The activity of the pyruvate dehydrogenase complex is the rate-limiting step in the oxidation of glucose in the brain, and is central to the normal function of this organ. It has been calculated [11] that the fully activated PDHC could oxidize 180 g of glucose each day; the brain normally

oxidizes 125 g a day, leaving little room for error caused by decreased activity of PDHC.

Among genetic deficiencies in PDHC, the most common result from mutations in the $E1\alpha$ gene on the X chromosome. The disease is expressed as an X-linked dominant. Abnormalities in $E1\beta$, protein X, $E3$, and PDH phosphatase are rare. The gene for $E1\alpha$ is at Xp22.1-22.2 [12–14]. The $E1\beta$ gene is on chromosome 3p13-q23 [13]. The $E3$ gene is on chromosome 7 at q31-32 [13, 15]. These three genes have been cloned and sequenced [16–18]. The gene for the X protein is on chromosome 11p [19]. A considerable number of mutations has been defined in the $E1\alpha$ gene [20]. Most of those in males have been missense mutations, while in females there have been major disruptions in the single affected X chromosome [21, 22].

CLINICAL ABNORMALITIES

Among the defined oxidative abnormalities that lead to lactic acidemia, deficiency of PDHC has been reported to be the most common [23, 24], although this certainly has not been our experience. We have seen many more

mitochondrial DNA defects and electron transport chain abnormalities. In a series of 54 patients with deficiency of E1 the spectrum of clinical presentation was as broad as those of the lactic acidemias in general (Chapter 46).

The most severe presentation is of neonatal or infantile metabolic acidosis with lactic acidemia. The acute neonatal presentation may be complicated by hyperammonemia. A majority of these infants die prior to six months of age, many of them in the neonatal period [25–28].

There is a somewhat more indolent presentation in patients with chronic, more modest lactic acidemia who first come to attention because of delayed psychomotor development [29–36]. Some may present with failure to thrive or poor linear growth. Many of these patients have the clinical and neuropathological features of Leigh syndrome [37]. As many as 25 percent of patients with Leigh syndrome have been reported to have defects of PDHC [24, 36]. Many of these patients die between ten months and three years of age. They may experience rapid deterioration or episodic deterioration following infections. Patients may have dystonia and ultimately they develop spastic quadripareisis. A variety of seizures includes grand mal, myoclonic, absence, or akinetic convulsions. With progression, brainstem abnormalities become prominent. There may be ocular movement abnormalities and central respiratory failure. Death may be from apnea or pneumonia. Another group of patients are characterized by ataxia and lactic acidosis [27].

Neuroimaging may reveal attenuated signal in the basal ganglia, particularly in putamen and globus pallidus [38, 39], and ultimately generalized cerebral atrophy. Histopathological examination reveals spongiform degeneration and gliosis especially in the basal ganglia. Among the neonatal acidosis group, some have had cortical cysts at autopsy [24, 39]. A number of these patients has also had agenesis of corpus callosum. Cerebral atrophy may be generalized.

The least severe presentations tend to be females with a slowly progressive Leigh syndrome or males with ataxia resembling a slowly progressive spinocerebellar degeneration [40]. Among the group with psychomotor impairment surviving at report, females outnumbered males 2:1 [36]. Among those with the ataxia presentation, some ataxia may be episodic, and some may be induced by carbohydrate and ameliorated by a high lipid diet [41, 42]. A rare presentation is with peripheral neuropathy of infantile origin associated with hypotonia and absent deep tendon reflexes [43]. Episodic weakness has been described in a patient with ataxia and impaired reflexes [11]. Some patients have elevated concentrations of lactic acid in cerebrospinal fluid (CSF) with little or no elevation in the blood [44, 45]; this has been referred to as cerebral lactic acidosis [45].

A group of patients has been described in which dysmorphic features signified prenatal onset of effects of deficiency of PDHC (Figures 49.2, 49.3, and 49.4) [24, 46–48]. Our two patients [46], who were siblings, displayed



Figure 49.2 LR: An infant with lactic acidemia and deficiency of the pyruvate dehydrogenase complex (PDHC). In addition, she had frontal bossing, a depressed nasal bridge, and an anteverted nasal tip. The ear was large and unusual in shape.



Figure 49.3 LR: The lower extremities were in extreme external rotation.



Figure 49.4 Infant with the pyruvate dehydrogenase complex (PDHC) deficiency [48], whose face and head had a similar appearance to the infant in Figure 49.2. (Illustration was kindly provided by Dr Richard Wennberg of the University of California, Davis.)

virtually complete absence of psychomotor development. Their phenotype was quite similar to the patient of Farrell and colleagues [48]. Characteristics were wide separation of the eyebrows, epicanthal folds, depressed nasal bridge, a small nose with anteverted flared nostrils (Figures 49.2 and 49.4), and a long philtrum. There was limited extension of the elbows and ulnar deviation of the hands. Both hips appeared to be dislocated. The position of the legs was in external rotation so that the feet pointed out like those of a ballerina (Figure 49.3), and abduction was very limited. There was a ventricular septal defect. Visual evoked potentials revealed only a very small degree of cortical response. The facial features have been considered to resemble those of the fetal alcohol syndrome [49]. A common mechanism suggested would be low fetal activity of PDHC, a result in the latter condition of inhibition by acetaldehyde.

GENETICS AND PATHOGENESIS

Deficiency of E1 α , the only common form of abnormality in PDHC, behaves as an X-linked dominant character in which, depending on the mutations, there may be quite severe disease in the female. All of the other defects are autosomal recessive. Reduced conversion of 1-¹⁴C-pyruvate to CO₂ has been demonstrated in the fibroblasts of parents consistent with heterozygosity [50], but in four obligate heterozygotes, assay of PDHC revealed somewhat lower than normal levels in two, and normal levels in two. The variability of enzyme assay makes prenatal diagnosis, as well as heterozygote detection, unreliable. In those families in which the mutation has been identified, prenatal diagnosis and heterozygote detection can be pursued with molecular methodology.

Deficiency of PDHC can be documented by assay of a variety of tissues; it is most often accomplished by the study of cultured fibroblasts [23, 24]. The simplest procedure is to measure the conversion of 1-¹⁴C-pyruvate to ¹⁴CO₂. Considerable variability has been the rule in the assay, and it has not been possible to correlate the amount of residual activity with the severity of the clinical phenotype. The substrate itself is unstable [51] and activity is also influenced by the methodology employed for disrupting the cell [52]. Assays generally take advantage of the use of dichloroacetate to inhibit PDH kinase and maximize the proportion of the active PDH enzyme [52–54]. Localization of the defect to the E1 component has generally been done [55] by incorporating ferricyanide into the reaction mixture as an artificial electron acceptor to oxidize the hydroxyethylthiamine and regenerate active E1 enzyme. Deficiency of activity of PDHC has been reported to range from 3 to 40 percent of the control level [23, 24].

There is a tendency for those with severe neonatal disease and dysmorphic features to have virtually no enzymatic activity [48], and for most patients with indolent disease to have considerably more residual enzyme activity [23],

but overall the correlation between survival and measured activity has not been good. In general, the severity of disease also correlates with the height of the lactic acidemia; in the fatal neonatal patients, it is very high. Studies of the kinetic characteristics of the enzyme in patients have been few. In one, the PDHC of autopsied liver was difficult to activate [56]. In another [11], the deficient enzyme in muscle biopsied during an acute attack was completely deactivated, although it could be activated *in vitro*.

Antisera have been prepared against purified E1, E2, and E3 [34]. In 19 of 22 patients with deficiency of PDHC in whom the defect was localized to E1, and cellular proteins were labeled with ³⁵S-methionine and immunoprecipitated by antibodies to E1, the deficiency of E1 activity was correlated with a deficient α -subunit [34]. Other patients with deficient E1 activity were immunochemically normal; consistent with simple amino acid substitution resulting in loss of activity. Visualization of PDH proteins by electrophoresis and immunoblotting or immunoprecipitation has revealed decreased or absent E1 α protein [36, 57–59], as well as altered migration and an increased phosphorylated form [27, 60]. Whenever E1 α is decreased, there is proportional decrease in E1 β . Complete absence of E1 α and E1 β may be associated with fatal neonatal acidosis [27, 36], or with impaired mental development and ataxia [34]. Females with deficient activity and clinical disease have been documented to have two E1 α bands [58, 59].

Cloning of the *E1 α* gene has permitted extensive documentation of the nature of mutation in this disease. The *E1 α* gene has 11 exons spanning 17 kb [16]. It is of interest that a majority of the mutations identified have been in exons 5 to 11; they include the region in which pyruvate is bound to the enzyme (amino acids 130–150), the TPP binding site (170–226), and the serine phosphorylation sites (231–291) [9, 20, 21, 60]. On the other hand, in another series, 12 of 20 mutations were found in exons 10 and 11 [21]. Females with E1 deficiency have two genes, one normal *E1 α* gene and one with the mutated gene.

Missense mutation in the pyruvate binding region, a substitution of methionine at position 138 for valine was found in two sisters with 0.6–7 percent enzyme activity and clinical mental impairment who died at 10 and 11 years [20]. Among mutations in the TPP binding area, an alanine-to-threonine 170 change led to a slowly progressive Leigh picture, while a phenylalanine 176-to-leucine change led to death in infancy with severe lactic acidosis [20, 21]. An alanine-to-threonine change at 231, just before the serine phosphorylation site at 232, led to 1.7 percent activity and death from lactic acidosis at 7 days [20]. Similarly, a histidine-to-leucine 263 change just before the serine 264 phosphorylation site gave only 2.5 percent residual activity in a female. An arginine 349-to-histidine mutation very close to the N terminus of the gene occurred in a male with brain atrophy and death at 13 weeks [61]. On the other hand, milder disease was observed in 13 unrelated patients with an arginine 234-to-glycine mutation [20, 62, 63],

and 50 percent residual activity was found in two females with an arginine 273-to-cysteine mutation [62]. A recently discovered tyrosine 243-to-serine change in a patient with neonatal lactic acidemia and bilateral globus pallidus lesions was correlated in fibroblasts *in vitro* with an absence of the normal increase in activity on addition of TPP [64].

Deletions and insertions have been reported in exons 10 and 11. Many of these patients had severe lactic acidosis and died early in life [61, 63, 65]. An exception was a CAGT deletion at 1167 that produced a protein with 33 extra amino acids and a clinical picture of only exercise intolerance [65]. An unusual mutation in *E1 α* [66], in a family with Leigh encephalomyelopathy presentations, led to alternate splicing in which all of exon 6 containing the TPP binding sites was lost in some transcripts. The mutation, an A-to-G substitution at position 660, did not change the glycine at this position, but led to the loss of exon 6. The mother exhibited normal activity and had 90 percent of normal alleles. A similar mutation in exon 8 of the *HPRT* gene led to classic Lesch-Nyhan disease (Chapter 65). In one of our patients whose mutation led to loss of the last three amino acids, onset was at 16 years, and at 35 years he displayed ataxia and dysarthria. He was also psychotic. A mutation (R20P) in a patient with Leigh syndrome has been identified in the mitochondrial targeting sequence, altering import of the precursor protein into the mitochondria [67].

In a study of immunoactive enzyme, mRNA, and mutation, three patterns were found: (1) immunodetectable α and β enzymes; (2) no cross-reactive material, but mRNA for both; and (3) absent proteins but deficiency of only α mRNA [62]. It was concluded that failure of expression of one mRNA led to instability of the entire complex. In a boy with microcephaly and developmental delay and a splice site mutation in the *E1 α* gene, somatic mosaicism was found with mixtures of normal and variant α enzyme [68].

Mutations in the *E1 β* gene were found in two unrelated patients [69]. In both, immunoreactive protein was decreased. Both had missense mutations c.395A>G (p.Y132C) and c.1030C>T (p.P344S). Both had severe neonatal onset disease.

Abnormalities in E2 or protein X have been reported in fewer than ten patients [70–72]. A clinical picture of severe neonatal lactic acidosis and hyperammonemia was associated with 24 percent residual activity of PDHC, and 32 percent transacetylase activity [70]. The E2 protein was absent and protein X was reduced. Absent protein X was found in a patient with severe psychomotor impairment, with lactic acidosis and 12 percent activity of PDHC. A boy with an extra band below protein X on immunoblotting with antibody to PDH which was found to be a variant of E2 had an initial presentation of ataxia without impaired mental development, but developed a neuroimaging picture of Leigh disease; fibroblasts displayed 55 percent of control activity of PDHC [70]. Severe lactic acidosis and absence of the corpus callosum occurred in an infant [71] with an absence of the X component. Two patients with a

Leigh syndrome presentation [72] had specific absence of the X protein.

Two unrelated consanguineous patients with episodic dystonia and lesions in the globus pallidus were each homozygous for mutations on the *DLAT* gene which codes for the E2 protein [73].

Component X binds to E3, and is also referred to as the E3-binding protein [74]. In two unrelated patients with homozygous splice site mutations and neonatal lactic acidosis, the mutations were a G>A mutation at the donor splice site of intron 5 leading to exon 5 deletion, and a G>A transition of the splice acceptor site of intron 8. Neonatal lactic acidosis, severe encephalopathy, and death at 35 days was reported in a girl with a homozygous deletion (620 delC) in the *PDX1* gene [75]. In a girl with a large 3913-bp deletion of the *PDX1* gene involving introns 9 and 10 and exon 10, the patient had developmental delay and spastic paraparesis, but was static until 14 years of age when she developed status epilepticus and was treated with valproate, which was followed by severe metabolic and neurologic decompensation [76]. A novel mechanism for the causation of human disease was found in a 25-year-old man with psychomotor delay, spastic diplegia. He developed recurrent dystonia, which disappeared with institution of a ketogenic diet [77]. He had a p.Q248X mutation on the paternal allele. On the maternal side, there was a 46-kb deletion combined with the integration of a full length LINE-1 element. A model of template jumping was suggested as the mechanism of retropositional insertion of the full-length element.

Defects in E3 (Chapter 50) result in deficiency of 2-oxoglutarate dehydrogenase and the branched-chain oxoacid decarboxylase, as well as PDHC. In these patients, lactic acidemia and systemic acidosis developed some months after birth. Elevated levels may be found of the branched-chain amino acids and of 2-oxoglutarate, as well as of pyruvate and lactate. In one patient, 2-oxoisocaproic acid was found in elevated amounts. Activity of lipoamide dehydrogenase ranged from 0 to 20 percent of the control level.

Among mutations reported in the *E3* gene, lysine 37 to glutamic acid and proline 453 to leucine were found on the two alleles in a patient with no detectable activity of E3 [78]. In a boy with microcephaly, impaired mental development, and lactic acidemia, along with recurrent hypoglycemia and ataxia, novel mutations were found in the *E3* gene [79]. They were p.I393T in exon 11 and IVS9+G>A. Pyruvate dehydrogenase activity in fibroblasts was normal.

Deficiency of pyruvate dehydrogenase phosphatase has been found in a few patients' gene [6, 80, 81] on chromosome 8q22-23 at nucleotide 716, leading to p.D239V in a patient who had congenital lactic acidemia and defective activation of pyruvate decarboxylase by removing phosphate from serine residues in *E1 α* [82]. Among other patients with deficiency of pyruvate dehydrogenase phosphatase, one died at six months of severe metabolic acidosis and lactic acidemia [6] and four had Leigh disease phenotypes [80, 81].

TREATMENT

Patients with deficiency of PDHC are sensitive to carbohydrate [41] and may develop life-threatening acidosis when given a diet high in carbohydrates. They respond to the administration of glucose with elevation in concentrations of pyruvate and lactate [41]. The provision of a diet low in carbohydrate and high in fat may lead to reduction in the concentration of lactate and some improvement in the general condition of the patient [26, 41, 83]. In one patient, a diet with 58–66 percent fat was followed by reversal of elevated concentrations of lactic acid and magnetic resonance imaging (MRI) evidence of improvement in the brain [32]. In another patient with E1 α mutations, treatment with a ketogenic diet led to reversal of T₂ hyperintensity in the globus pallidus [84]. Levels of lactate in the blood may be brought within the normal range using diets in which 50 percent or more of the calories are in fat and 20 percent in carbohydrate. These diets may lead to ketonemia or ketonuria, but not to acidosis or hypoglycemia.

Despite clinical improvement attendant on amelioration of acidotic symptoms, patients with neurologic abnormalities do not usually improve neurologically. These diets bypass the defect by providing the product of the PDHC reaction directly as acetylCoA from the metabolism of fat. In addition, the lesser load of carbohydrate provides smaller amounts of pyruvate to accumulate behind the block and cause lactic acidosis. Caveats raised concerning the use of these diets [85] included the possibility that they could be high in protein and could cause hypercalciuria or kidney stones, as have been observed in patients with convulsions treated with ketogenic diets [86, 87], but the only potentially adverse effect observed in the patients with deficiency of PDHC reviewed was hyperuricemia.

Since thiamine pyrophosphate is an integral component of the E1 enzyme, high doses of thiamine (100–600 mg/day) have been employed in the hope that a decreased affinity for the cofactor could be overcome by increasing its concentration. Improvement has been reported in a patient described as thiamine-dependent [25]. In other patients, a trial of thiamine is worthwhile in the hope of stimulating residual activity of pyruvate decarboxylase.

Dichloroacetate (Chapter 46) effectively reduces levels of lactate in most patients, consistent with the presence of residual activity in PDH in most of them. Intuitively, one might not expect to achieve much in the way of clinical improvement in neurological features of this disease by treatment with DCA, but we have encountered some dramatic improvements in some patients with PDH deficiency. Responsiveness to DCA in cultured fibroblasts has been correlated with genotype in severe E1 α deficiency [88]. Appreciable increase in PDHC activity in the presence of DCA was found in cell lines with R378C and R88C mutations, consistent with reduced degradation of polypeptides with reduced stability. Carnitine and coenzyme Q are employed in many centers in the treatment

of patients with deficiency of PDHC. The usual dose of coenzyme Q is 4 mg/kg, although we are now measuring levels of coenzyme Q, and in deficient patients, often those with electron transport defects, we have employed 10–20 mg/kg, and some use considerably more.

In the management of the acute episode of lactic acidosis (Chapter 46), the usual approach is to provide large quantities of intravenous water and electrolytes in the form of NaHCO₃. Stacpoole [89] has argued a case against the use of bicarbonate in lactic acidosis, at least in those adult patients with secondary lactic acidemia in intensive care units (ICUs). He cited evidence that infused bicarbonate forms carbon dioxide, which may diffuse across the blood–brain barrier, lowering the pH of cerebrospinal fluid; as well as evidence, in experimental lactic acidosis in animals of decreased cardiac output and increased intestinal formation of lactic acid with bicarbonate infusion as opposed to saline infusion. However, these experiments studied the hyperosmolar 1 molar NaHCO₃ solution used in ICUs compared with isotonic solutions of NaCl. They may provide an argument for the use of isotonic NaHCO₃ in the management of acidosis.

REFERENCES

1. Reed LJ, Pettit FH, Yeaman SJ *et al.* Structure function and regulation of the mammalian pyruvate dehydrogenase complex. *Proc Eur J Biochem Soc* 1980; **60**: 47.
2. Matuda S, Kitano A, Sakaguchi Y *et al.* Pyruvate dehydrogenase complex with lipoamide dehydrogenase deficiency in a patient with lactic acidosis and branched chain ketoaciduria. *Clin Chim Acta* 1984; **140**: 59.
3. De Marcucci GL, Hodgson JA, Lindsay G. The Mr 50000 polypeptide of mammalian pyruvate dehydrogenase complex participates in acetylation reactions. *Eur J Biochem* 1986; **158**: 587.
4. Powers-Greenwood SL, Rahmatullah M, Radke GA, Roche TE. Separation of protein X from the dihydrolipoyl transacetylase component of the mammalian pyruvate dehydrogenase complex and function of protein X. *J Biol Chem* 1989; **264**: 3655.
5. Neagle JC, Lindsay JG. Selective proteolysis of the protein X subunit of the bovine heart pyruvate dehydrogenase complex. *Biochem J* 1991; **278**: 423.
6. Robinson BH, Sherwood WG. Pyruvate dehydrogenase phosphatase deficiency: a cause of congenital chronic lactic acidosis in infancy. *Pediatr Res* 1975; **9**: 935.
7. Randle PJ, Sugden PH, Kerbey AL *et al.* Regulation of pyruvate oxidation and the conservation of glucose. *Biochem Soc Symp* 1979; **43**: 67.
8. Whitehouse S, Cooper RH, Randle PJ. Mechanism of activation of pyruvate dehydrogenase by dichloroacetate and other halogenated carboxylic acids. *Biochem J* 1974; **141**: 671.
9. Randle PJ. Mitochondrial 2-oxoacid dehydrogenase complexes of animal tissues. *Philos Trans* 1987; **302**: 47 (Abstr.).

10. Ahlborg O, Felig P. Lactate and glucose exchange across the forearm legs and splanchnic bed during and after prolonged leg exercise. *J Clin Invest* 1982; **68**: 45.
11. Robinson BH, Sherwood WG. Lactic acidemia the prevalence of pyruvate decarboxylase deficiency. *J Inherit Metab Dis* 1984; **7**: 69.
12. Brown RM, Dahl H-HM, Brown GK. X chromosome localization of the functional gene for E1 α subunit of the human pyruvate dehydrogenase complex. *Genomics* 1989; **7**: 215.
13. Olson S, Song BJ, Hueh TL *et al*. Three genes for enzymes of the pyruvate dehydrogenase complex map to human chromosomes 3, 7 and X. *Am J Hum Genet* 1990; **46**: 340.
14. Szabo P, Sheu KFR, Robinson RM *et al*. The gene for alpha polypeptide of pyruvate dehydrogenase is X-linked in humans. *Am J Hum Genet* 1990; **46**: 874.
15. Sherer SW, Otulakowski G, Robinson BH, Tsui L-C. Localization of the human dihydrolipoamide dehydrogenase gene (DLD) to 7q31-q32. *Cytogenet Cell Genet* 1991; **56**: 176.
16. Maragos C, Hutchison W, Haysaka K *et al*. Structural organization of the gene for the E1 α subunit of the human pyruvate dehydrogenase complex. *J Biol Chem* 1989; **26**: 12294.
17. Koike K, Urata Y, Koike M. Molecular cloning and characterization of human pyruvate dehydrogenase β subunit gene. *Proc Natl Acad Sci USA* 1990; **87**: 5594.
18. Feigenbaum A, Robinson BH. Structural organization of the human lipoamide dehydrogenase gene. *Genomics* 1993; **17**: 376.
19. Aral B, Benelli C, Ait-Ghezala G *et al*. Mutations in PDX1 the human lipoyl-containing component X of the pyruvate dehydrogenase-complex gene on chromosome 11p1 in congenital lactic acidosis. *Am J Hum Genet* 1997; **61**: 1318.
20. Chun K, MacKay N, Petrova-Benedict R, Robinson BH. Mutations in the X-linked E1 α subunit of pyruvate dehydrogenase leading to deficiency of the pyruvate dehydrogenase complex. *Hum Mol Genet* 1993; **2**: 449.
21. Dahl H-HM, Brown GK, Brown RM *et al*. Mutations and polymorphisms in the pyruvate dehydrogenase E1 α gene. *Hum Mutat* 1992; **1**: 97.
22. Dahl H-HM, Maragos C, Brown RM *et al*. Pyruvate dehydrogenase deficiency caused by deletion of a 7bp repeat sequence in the E1 α gene. *Am J Hum Genet* 1990; **47**: 286.
23. Robinson BH, Taylor J, Sherwood WG. The genetic heterogeneity of lactic acidosis: occurrence of recognizable inborn errors of metabolism in a pediatric population with lactic acidosis. *Pediatr Res* 1980; **14**: 956.
24. Robinson BH, MacMillan H, Petrova-Benedict R, Sherwood WG. Variable clinical presentation in patients with defective E1 component of pyruvate dehydrogenase complex. A review of 30 cases with a defect in the E1 component of the complex. *J Pediatr* 1987; **111**: 525.
25. Wick H, Schweizer K, Baumgartner R. Thiamine dependency in a patient with congenital lactic acidemia due to pyruvate dehydrogenase deficiency. *Agents Actions* 1977; **7**: 405.
26. Strömme JH, Borud O, Moe PJ. Fatal lactic acidosis in a newborn attributable to a congenital defect of pyruvate dehydrogenase. *Pediatr Res* 1976; **10**: 60.
27. Brown GK, Otero LJ, LeGris M *et al*. Pyruvate dehydrogenase deficiency. *J Med Genet* 1994; **31**: 875.
28. Matsuo M, Ookita K, Takemine H *et al*. Fatal case of pyruvate dehydrogenase deficiency. *Acta Paediatr Scand* 1985; **74**: 140.
29. Evans OB. Pyruvate decarboxylase deficiency in subacute necrotizing encephalomyelopathy. *Arch Neurol* 1981; **38**: 515.
30. Papanastasiou D, Lehnert W, Schuchmann L, Hommes FA. Chronic lactic acidosis in an infant. *Helv Paediatr Acta* 1980; **35**: 253.
31. Hansen TL, Christensen E, Brandt NJ. Studies on pyruvate carboxylase pyruvate decarboxylase and lipoamide dehydrogenase in subacute necrotizing encephalomyelopathy. *Acta Paediatr Scand* 1982; **71**: 263.
32. Toshima K, Kuroda Y, Hashimoto T *et al*. Enzymologic studies and therapy of Leigh's disease associated with pyruvate decarboxylase deficiency. *Pediatr Res* 1982; **16**: 430.
33. Miyabayashi S, Ito T, Narisawa K *et al*. Biochemical studies in 28 children with lactic acidosis in relation to Leigh's encephalomyelopathy. *Eur J Pediatr* 1985; **143**: 278.
34. Ho L, Hu CWC, Packman S *et al*. Deficiency of the pyruvate dehydrogenase component in pyruvate dehydrogenase complex-deficient human fibroblasts. Immunological identification. *J Clin Invest* 1986; **78**: 844.
35. Ohtake M, Takada G, Miyabayashi S *et al*. Pyruvate decarboxylase deficiency in a patient with Leigh's encephalomyelopathy. *Tohoku J Exp Med* 1982; **137**: 379.
36. Robinson BH, Chun K, MacKay N *et al*. Isolated and combined deficiencies of the α -keto acid dehydrogenase complexes. *Ann NY Acad Sci* 1989; **573**: 337.
37. Leigh D. Subacute necrotizing encephalomyelopathy in an infant. *J Neurol Neurosurg Psychiatry* 1972; **14**: 87.
38. Hall K, Gardner-Medwin D. CT scan appearances in Leigh's disease (subacute necrotizing encephalomyelopathy). *Neuroradiology* 1978; **16**: 48.
39. Medina L, Chi TL, DeVivo DC, Hilal SK. MR findings in patients with subacute necrotizing encephalomyelopathy (Leigh syndrome): correlation with biochemical defect. *Am J Roentgenol* 1990; **154**: 1269.
40. Blass JP, Lonsdale D, Uhlenhuth BW, Hom E. Intermittent ataxia with pyruvate decarboxylase deficiency. *Lancet* 1971; **1**: 1302.
41. Cederbaum SD, Blass JP, Minkoff N *et al*. Sensitivity to carbohydrate in a patient with familial intermittent lactic acidosis and pyruvate dehydrogenase deficiency. *Pediatr Res* 1976; **10**: 713.
42. Blass JP, Schulman JD, Young DS, Hom E. An inherited defect affecting the tricarboxylic acid cycle in a patient with congenital lactic acidosis. *J Clin Invest* 1972; **51**: 1845.
43. Chabrol B, Mancini J, Benelli C. Leigh syndrome: pyruvate dehydrogenase defect. A case with peripheral neuropathy. *J Child Neurol* 1994; **9**: 52.
44. Brown GK, Brown RM, Scholem RD *et al*. The clinical and biochemical spectrum of human pyruvate dehydrogenase complex deficiency. *Ann NY Acad Sci* 1989; **573**: 360.
45. Brown GK, Haan EA, Kirby DM *et al*. 'Cerebral' lactic acidosis: defects in pyruvate metabolism with profound brain damage and minimal systemic acidosis. *Eur J Pediatr* 1988; **147**: 10.

46. Nyhan WL, Sakati NA. Pyruvate dehydrogenase deficiency. In: *Diagnostic Recognition of Metabolic Disease*. Philadelphia: Lea and Febiger, 1987: 228.
47. Sherwood WG, Robinson BH. Dysmorphism in congenital lactic acidosis syndrome. *Pediatr Res* 1984; **18**: 300A.
48. Farrell DF, Clark AF, Scott CR, Wennberg RP. Absence of pyruvate decarboxylase activity in man: a cause of congenital lactic acidosis. *Science* 1975; **187**: 1082.
49. Jones KL, Smith DW, Ulleland CW, Streissguth AP. Pattern of malformation in offspring of chronic alcoholic mothers. *Lancet* 1973; **1**: 1267.
50. Robinson BH. Inborn errors of pyruvate metabolism. *Biochem Soc Trans* 1983; **11**: 623.
51. Silverstein E, Boyer PD. Instability of pyruvate ^{14}C in aqueous solutions as detected by enzymic assay. *Anal Biochem* 1964; **8**: 470.
52. Haas RH, Thompson J, Morris B *et al.* Pyruvate dehydrogenase activity in osmotically-shocked rat brain mitochondria: stimulation by oxaloacetate. *J Neurochem* 1988; **50**: 673.
53. Sheu KF, Hu CC, Utter MF. Pyruvate dehydrogenase complex activity in normal and deficient fibroblasts. *J Clin Invest* 1981; **67**: 1463.
54. Johnston K, Newth CJL, Sheu K-FR *et al.* Central hypoventilation syndrome in pyruvate dehydrogenase complex deficiency. *Pediatrics* 1984; **74**: 1034.
55. Reed LJ, Willms CR. Purification and resolution of the pyruvate dehydrogenase complex (*Escherichia coli*). *Meth Enzymol* 1966; **9**: 247.
56. MacKay N, Petrova-Benedict R, Thoene J *et al.* Three cases of lactic acidemia due to pyruvate decarboxylase (E1) deficiency with evidence of protein polymorphism in the α subunit of the enzyme. *Eur J Pediatr* 1986; **144**: 445.
57. Old SE, DeVivo DC. Pyruvate dehydrogenase complex deficiency: biochemical and immunoblot analysis of cultured skin fibroblasts. *Ann Neurol* 1989; **26**: 746.
58. Endo H, Miyabashi S, Toda K, Narisawa K. A four-nucleotide insertion at the E1a gene in a patient with pyruvate dehydrogenase deficiency. *J Inherit Metab Dis* 1991; **14**: 793.
59. Kitano A, Endo F, Matsuda I. Immunochemical analysis of pyruvate dehydrogenase complex in two boys with primary lactic acidemia. *Neurology* 1990; **40**: 1312.
60. Hawkins CF, Borges A, Perham RN. A common structural motif in thiamine pyrophosphate-binding enzymes. *FEBS Lett* 1989; **255**: 77.
61. Hansen LL, Brown GK, Kirby DM, Dahl H-HM. Characterization of the mutations in three patients with pyruvate dehydrogenase E1 α deficiency. *J Inherit Metab Dis* 1991; **14**: 140.
62. Wexler ID, Kerr DS, Ho L *et al.* Heterogeneous expression of protein and RNA in pyruvate dehydrogenase deficiency. *Proc Natl Acad Sci USA* 1988; **85**: 7336.
63. Dahl H-HM, Maragos C, Brown RM *et al.* Pyruvate dehydrogenase deficiency caused by a 7 bp repeat sequence in the E1 β gene. *Am J Hum Genet* 1990; **47**: 286.
64. Benelli C, Fouque F, Redonnet-Vernhet I *et al.* A novel Y243S mutation in the pyruvate dehydrogenase E1 α gene subunit: correlation with thiamine pyrophosphate interaction. *J Inherit Metab Dis* 2002; **25**: 325.
65. Endo H, Hasegawa K, Narisawa K *et al.* Defective gene in lactic acidosis: abnormal pyruvate dehydrogenase E1 α -subunit caused by a frameshift. *Am J Hum Genet* 1989; **44**: 358.
66. De Meirleir L, Lissens W, Benelli C *et al.* Aberrant splicing of exon 6 in the pyruvate dehydrogenase-E1 α mRNA linked to a silent mutation in a large family with Leigh's encephalomyelopathy. *Pediatr Res* 1994; **36**: 707.
67. Takakudo F, Cartwright P, Hoogenraad N *et al.* An amino acid substitution in the pyruvate dehydrogenase E1 α gene affecting mitochondrial import of the precursor protein. *Am J Hum Genet* 1995; **57**: 772.
68. Okajima K, Warman ML, Byrne LC *et al.* Somatic mosaicism in a male with an exon skipping mutation in PDHA1 of the pyruvate dehydrogenase complex results in a milder phenotype. *Mol Genet Metab* 2006; **87**: 162.
69. Brown RM, Head RA, Boubriak II *et al.* Mutations in the gene for the E1 β subunit: a novel cause of pyruvate dehydrogenase deficiency. *Hum Genet* 2004; **115**: 123.
70. Robinson BH, MacKay N, Petrova-Benedict R *et al.* Defects in the E2 lipoyl transacetylase and the X-lipoyl containing component of the pyruvate dehydrogenase complex in patients with lactic acidemia. *J Clin Invest* 1990; **85**: 1821.
71. Geoffroy V, Fouque F, Benelli C *et al.* Defect in the X-lipoyl-containing component of the pyruvate dehydrogenase complex in a patient with a neonatal lactic acidemia. *Pediatrics* 1996; **97**: 267.
72. Marsac C, Stansbie D, Bonne G *et al.* Defect in the lipoyl-bearing protein X subunit of the pyruvate dehydrogenase complex in two patients with encephalomyelopathy. *J Pediatr* 1993; **123**: 915.
73. Head RA, Brown RM, Zokipli Z *et al.* Clinical and genetic spectrum of pyruvate dehydrogenase deficiency: dihydrolipoamide acetyltransferase (E2) deficiency. *Ann Neurol* 2005; **58**: 234.
74. Brown RM, Head RA, Brown GK. Pyruvate dehydrogenase E3 binding protein deficiency. *Hum Genet* 2002; **110**: 187.
75. Dey R, Mine M, Desquerre I *et al.* A new case of pyruvate dehydrogenase deficiency due to a novel mutation in the PDX1 gene. *Ann Neurol* 2003; **53**: 273.
76. Schiff M, Mine M, Brivet M *et al.* Leigh's disease due to a new mutation in the PDHX gene. *Ann Neurol* 2006; **59**: 709.
77. Mine M, Chen JM, Brivet M *et al.* A large genomic deletion in the PDHX gene caused by the retrotranspositional insertion of a full-length LINE-1 element. *Hum Mutat* 2007; **28**: 137.
78. Liu T-C, Kim H, Arijmendi C *et al.* Identification of two missense mutations in a dihydrolipoamide dehydrogenase deficient patient. *Proc Natl Acad Sci USA* 1993; **90**: 5186.
79. Grafakou O, Oexie K, van den Heuvel L *et al.* Leigh syndrome due to compound heterozygosity of dihydrolipoamide dehydrogenase gene mutations. Description of the first E3 splice site mutation. *Eur J Pediatr* 2003; **162**: 714.
80. DeVivo DC, Haymond MW, Obert KA *et al.* Defective activation of the pyruvate dehydrogenase complex in subacute necrotizing encephalomyelopathy (Leigh disease). *Ann Neurol* 1979; **6**: 483.

81. Sorbi S, Blass JP. Abnormal activation of pyruvate dehydrogenase in Leigh disease fibroblasts. *Neurology* 1982; **32**: 555.
82. Shinahara K, Ohigashi I, Ito M. Cloning of a cDNA for human pyruvate dehydrogenase phosphatase and detection of a mutation in a patient with congenital lactic acidemia. *Am J Genet* 2000; **67**: 294.
83. Falk RE, Cederbaum SD, Blass JP *et al.* Ketogenic diet in the management of pyruvate dehydrogenase deficiency. *Pediatrics* 1976; **58**: 713.
84. Saenz MS, Pickler L, Elias E *et al.* Resolution of lesions on MRI with ketogenic diet in pyruvate dehydrogenase deficiency. *Mol Genet Metab* 2010; **99**: 187.
85. Weber TA, Antognetti R, Stacpoole PW. Caveats when considering ketogenic diets for the treatment of pyruvate dehydrogenase complex deficiency. *J Pediatr* 2001; **138**: 390.
86. Kinsman SL, Vining EPG, Quaskey SA *et al.* Efficacy of the ketogenic diet for intractable seizure disorders: review of 58 cases. *Epilepsia* 1992; **33**: 1132.
87. Chesney DC, Brouhard BH, Wyllie E, Powaski K. Biochemical abnormalities of the ketogenic diet in children. *Clin Pediatr* 1999; **38**: 107.
88. Fouque F, Brivet M, Boutron A *et al.* Differential effect of DCA treatment on the pyruvate dehydrogenase complex in patients with severe PDHC deficiency. *Pediatr Res* 2003; **53**: 793.
89. Stacpoole PW. Lactic acidosis: the case against bicarbonate therapy. *Ann Intern Med* 1986; **105**: 276.

Lactic acidemia and defective activity of pyruvate, 2-oxoglutarate, and branched chain oxoacid dehydrogenases

Introduction	368	Treatment	372
Clinical abnormalities	369	References	372
Genetics and pathogenesis	371		

MAJOR PHENOTYPIC EXPRESSION

Potentially lethal disorder of infancy, failure to thrive, hypotonia, metabolic acidosis, ketonuria, lactic acidosis, 2-oxoaciduria, and deficient activity of the three dehydrogenases for pyruvate, 2-oxoglutarate, and branched-chain 2-oxoacids.

INTRODUCTION

A small number of infants has been reported with a disorder in which severe lactic acidosis has been associated with excretion of large quantities of citric acid cycle intermediates, and there is defective activity of the pyruvate dehydrogenase complex (PDHC) (Figures 50.1, 50.2, and 50.3) and the other dehydrogenases involved in oxidative decarboxylations [1–8]. Most have died in infancy [2]. In most, defective activity of lipoamide dehydrogenase

(E₃) was reported or presumed [4–7]. In one the activity of E₃ was normal, but improved catabolism of branched-chain amino acids after the growth of fibroblasts in medium supplemented with lipoic acid suggested a defect in an enzyme catalyzing the attachment of lipoic acid to a component of the enzyme complexes [8]. The gene for lipoamide dehydrogenase has been assigned to chromosome 7q31-q32 [9]. A few mutations in the gene have been reported [10, 11].

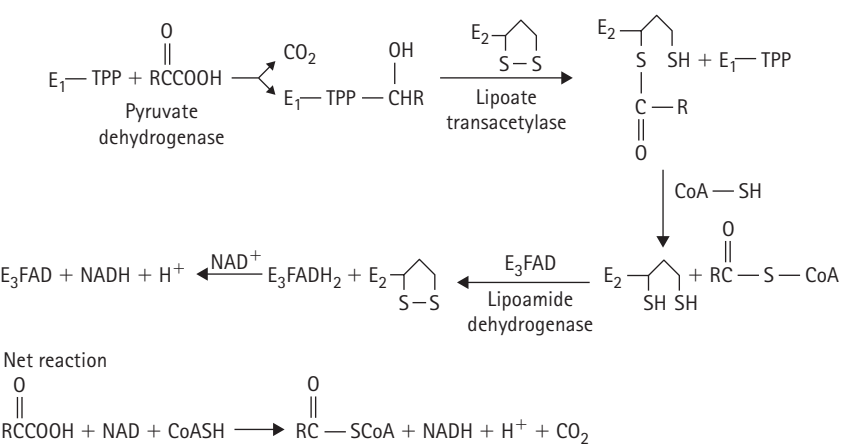


Figure 50.1 The pyruvate dehydrogenase complex. E₁ refers to pyruvate decarboxylase, E₂ to dihydrolipoyltransacetylase, and E₃ to lipoamide dehydrogenase. Lipoic acid (Figure 50.2) is shown already attached to E₂ (Figure 50.3).

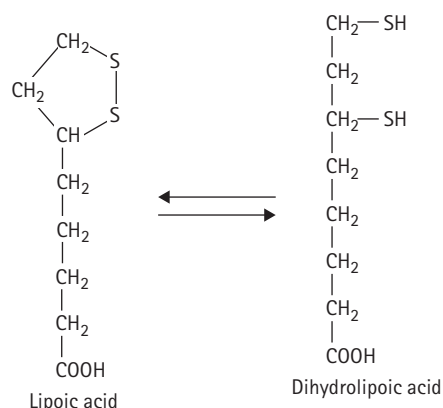


Figure 50.2 Lipoic acid in its oxidized and reduced forms. The interaction is catalyzed by E₃.

CLINICAL ABNORMALITIES

Despite the overwhelming nature of the illness, most patients have had an initial period of relative health that may have lasted as long as five months [8]. The first patient characterized by Robinson and colleagues in 1977 [1] was well until he became acutely ill at 8 weeks of age. He appeared pale and mottled and had labored respirations. He was lethargic and hypotonic. Failure to follow a light was consistent with bilateral optic atrophy. He had metabolic acidosis with a pH of 7.22 and a bicarbonate of 13 mEq/L. Hypoglycemia was observed on one occasion. Neurologic dysfunction was described as progressive and unremitting. He died following an aspiration at seven months.

Among the earliest reports was that of an Indian family from Canada in which three infants had lactic acidosis, impaired mental development, and seizures, and two excreted large amounts of pyruvic and 2-oxoglutaric acids in the urine [2]. Cultured fibroblasts derived from one sibling were found to be defective in pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase. Two died at three and four months, while the third was alive at report at 23 months. These patients developed acidosis very early in infancy. We also studied a two-year-old girl with lactic acidosis and severely impaired growth and mental development in whom the activities of PDHC and the 2-oxoglutarate dehydrogenase complex were deficient [3]. A previous sibling had died of what appeared to be the same syndrome.

In another patient, hypotonia, a poor Moro response, left esotropia, and an 'odd' cry were noted as early as 1 hour of life [4]. At 10 weeks, she developed vomiting and diarrhea and 12 hours later had a respiratory arrest. She was found to have slight hepatomegaly and a serum bicarbonate of 8 mEq/L. The blood glucose was 18 mg/dL and the lactate 8.8 mol/L. She had repeated episodes of ketoacidosis and dehydration that required admission to hospital and parenteral fluid therapy. After a second respiratory arrest at 17 months, she underwent considerable neurologic deterioration, but neurologic worsening had appeared to be a consequence of each of her episodes of acute illness; by ten months her suck was so poor a gastrostomy was placed. After 17 months, she no longer responded to verbal stimuli. Seizures occurred during the second year of life.

A Japanese infant failed to thrive and fed poorly for the first four months of life [5]. This was true of our patient too [8]. The Japanese infant developed pallor and tachypnea at six months of age and was found to have a blood pH of 7.17 and a bicarbonate of 7.4 mEq/L; the blood sugar was 38 mg/dL. Neurologic features at that time were hypotonia, poor head control, and dystonic movements. The developmental quotient was 78. By 17 months, he had spastic quadriplegia and nystagmus. Computed tomography (CT) scan revealed lucent lesions in the basal ganglia. He died during a ketoacidotic attack at 21 months. A Tunisian infant in France [6] also fed poorly and failed to thrive from the first week and by six months had hypotonia and severe developmental delay, but she did not become acutely ill until eight months, when she had an attack of acidosis in which the pH was 7.1, the bicarbonate was 5.51 mEq/L, and there was pronounced ketonuria and lactic acidemia (10 mmol/L). She died at 18 months in an episode of severe acidosis that was precipitated by an open biopsy of the liver. Another patient failed to gain weight from the neonatal period and had recurrent episodes of vomiting and metabolic acidosis [7]. At eight months of age, he weighed only 4.1 kg. He had hypotonia, poor muscle mass, and a pronounced head lag. CT scan revealed moderate cortical atrophy. The pH ranged from 7.0 to 7.2.

Our patient (Figure 50.4) was admitted at eight months for evaluation of lactic acidosis [8]. He had not developed acute symptomatic acidosis until 2 weeks prior to admission, although he had appeared weak, sucked poorly, and gained weight slowly from birth. After this long relatively benign early period, it was thereafter difficult

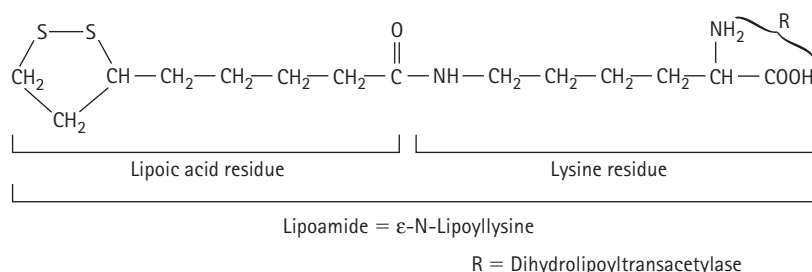


Figure 50.3 The structural attachment of lipoic acid to a lysine residue to E₂. This is reminiscent of the molecular attachment of biotin to the carboxylase apoenzymes.



Figure 50.4 EB: At ten months of age, two months after admission. He was symmetrically small and at the time had hypoproteinemia and edema.

to keep him out of the intensive care unit. The parents were first cousins. He was found to have a low serum bicarbonate and large amounts of lactic acid in blood and urine, and he was transferred to San Diego receiving 12 mEq/kg NaHCO_3 daily. The serum concentration of bicarbonate was 14 mEq/L. The blood concentration of lactate was 8.3 mmol/L and the pyruvate 0.34 mmol/L. Withdrawal of supplemental NaHCO_3 was followed by a decrease of the serum concentration of bicarbonate to 5 mEq/L. Analysis of the amino acid concentrations of the plasma revealed an elevated alanine ranging from 685 to 1000 mmol/L. Analysis of the amino acids of the urine revealed a generalized aminoaciduria. Further evidence of proximal renal tubular acidosis was a persistent urinary pH approximating 8.5 and 1–31 glycosuria. Proteinuria of 1–31 was also present. On admission, his urine tested strongly positive for ketones. Analysis of the organic acids of the urine revealed large amounts of lactate, acetoacetate,

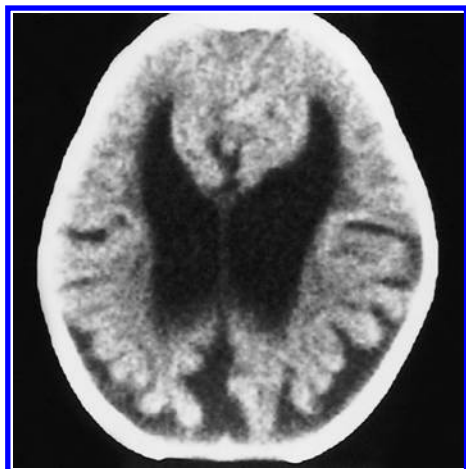


Figure 50.5 EB: Computed tomography scan at ten months. There was appreciable cerebral atrophy.

and 3-hydroxybutyrate. Electroencephalograph (EEG) revealed focal dysrhythmia in the left temporal region. CT scan (Figure 50.5) revealed evidence of cerebral atrophy.

Treatment was initiated with supplemental NaHCO_3 , but his condition worsened progressively and his bicarbonate requirement increased. Ultimately, it proved to be impossible to raise his serum concentration of bicarbonate without parenteral bicarbonate. Urinary obligatory water losses were such that he required 200–300 mL/kg per day and 60 mEq/kg NaHCO_3 were required to achieve a normal serum level of bicarbonate. It was impossible to provide sufficient protein and calories to permit adequate growth without ketonuria and increasing acidosis or diarrhea. He died seven months after admission.

Two patients [7, 8] had renal tubular acidosis. We have observed renal tubular acidosis in a number of patients with organic acidemia. Early death has been almost the rule, but some patients have had progressive neurologic deterioration. In some, the picture has been that of Leigh encephalopathy [12]. Neuropathology was that of myelin loss and cavitation in the basal ganglia [4].

Two patients have been reported [13, 14] with a milder phenotype (Figure 50.6) and normal cognitive function. One [13] had some motor problems and one had hepatocellular disease [14].



Figure 50.6 FM: A seven-year-old boy with a diagnosis of E_3 deficiency. At this time, he looked normal, but he had episodic attacks of vomiting progressive to lethargy and lactic acidosis. Each responded to fluid and electrolyte therapy. Tandem mass spectrometry of the blood was normal between attacks, but during the attack there was mild elevation of the branched-chain amino acids.

The acute acidotic episode in these patients differed from those of other patients with lactic acidemia because of the presence of pronounced ketonuria. This is reminiscent of the pattern in patients with pyruvate carboxylase deficiency (Chapter 47). Some patients have had hypoglycemia [1, 4, 5]. They have all had elevated concentrations of lactic acid and pyruvate in the blood. The characteristic urinary organic acid pattern was that of elevated excretion of lactate pyruvate and 2-oxoglutarate [5, 6, 8]. Elevated concentrations of 2-oxoglutarate have also been documented in the blood [1]. Other citric acid cycle intermediates, such as citrate, fumarate, and malate, may be found in increased amounts in urine. Excretion of 3-hydroxybutyrate may be massive [8] and there may be secondary elevation of 2-hydroxyisovalerate, as in any patient with ketosis. Elevated amounts of 2-hydroxyglutarate and 2-oxoisocaproate have also been reported in urine [15]. The plasma and/or urinary levels of alanine are often elevated [4, 6, 8]. In some patients, somewhat elevated concentrations of the branched-chain amino acids isoleucine, leucine, and valine have been recorded [1, 5, 14] and alloisoleucine may be found. In one patient [5], a neonatal screen for leucine revealed an elevated concentration in the blood.

GENETICS AND PATHOGENESIS

Defective enzyme activity has regularly been observed in the case of the pyruvate dehydrogenase complex and 2-oxoglutarate dehydrogenase [1, 4–8]. The assay has usually been carried out in cultured fibroblasts, but deficiency has also been demonstrated in a number of tissues, including liver [1, 5]. Levels of activity for the two enzymes ranged from 10 and 1 percent, respectively, of control to 38 and 39 percent of control [1, 4–8]. The activity of 2-oxocaproate dehydrogenase, 2-oxo-3-methylvalerate dehydrogenase, and 2-oxoisovalerate dehydrogenase have also been documented to be deficient, as have the oxidation of leucine and valine to CO_2 [8]. Thus, there is a generalized defect in these patients in the enzymes involved in oxidative decarboxylation.

Oxidative decarboxylation is typified by the pyruvate dehydrogenase complex (Figure 50.1), but it is also the function of 2-oxoglutarate dehydrogenase and branched-chain amino acid dehydrogenase. The only enzyme known to be shared by the three enzyme complexes is lipoamide dehydrogenase (dihydrolipoyl dehydrogenase) (E_3) (EC 1.6.4.4.3) (Figure 50.2). The pyruvate dehydrogenase complex consists of five known enzymatic components. Those that catalyze the actual decarboxylation include pyruvate decarboxylase (E_1) (EC 4.1.1.1) and dihydrolipoyl transacetylase (E_2) as well as E_3 . E_1 exists in inactive and active forms, or phosphorylated and dephosphorylated proteins, respectively, and the activation is catalyzed by a phosphatase and the inactivation by a kinase.

An enzymatic process catalyzing the attachment of

lipoic acid to the E_2 enzyme (Figure 50.3) is not known. However, it would be surprising if one did not exist. The covalent bond formed between the carboxyl group of lipoic acid and the amino group of a lysine residue of the E_2 protein would require energy. The structure suggests by analogy the attachment of biotin to the apocarboxylases propionyl CoA carboxylase, 3-methylcrotonyl CoA carboxylase, and pyruvate carboxylase, which is catalyzed by holocarboxylase synthetase, the enzyme that is deficient in the neonatal form of multiple carboxylase deficiency.

Deficiency of lipoamide dehydrogenase (E_3) has been documented in a number of patients [1, 4–7, 15]. In the first patient, activity of 5–10 percent of control was observed in a variety of tissues obtained post mortem [1]. In another, activity in cultured fibroblasts was 5 percent [4], while no activity could be found in biopsied liver or muscle. In another, activity was undetectable in either fibroblasts or liver [5, 14]. In one patient with E_3 deficiency, the residual activity of the enzyme in fibroblasts was 20 percent of the control mean and the kinetics of the enzyme in both forward and reverse directions were normal [7]. This was a patient who was reported to respond to lipoic acid and it is of interest that he was the one with the substantial amount of activity. However, *in vitro* addition of lipoic acid to the incubation mixtures did not increase activity of PDHC or ketoglutarate dehydrogenase complex (KGDHC). One of the patients with normal cognitive function had 12 percent of residual E_3 activity [12]. Immunochemical studies with antibody to porcine E_3 enzyme revealed normal amounts of enzyme protein in fibroblasts of each of three patients studied [16].

E_3 deficiency and the other forms of combined or multiple oxoacid dehydrogenase deficiency appear to be transmitted by rare autosomal recessive genes. Consanguinity has been reported [1, 8]. In another family of Canadian Indians, there were three affected siblings, a girl and two boys, and the parents were thought to be consanguineous [2].

The E_3 enzyme, as isolated from porcine heart is a homodimer with a molecular weight of 160 kDa [16]. The human gene has 14 exons [17]. The cDNA codes for an enzyme with homology with glutathione reductase [18]. A number of mutations has been identified. They include K37E and P453L, found in compound heterozygous Japanese patients [19] and G229C, a frequent allele in Ashkenazi Jews [20]. A patient with A1173G/del455-457 had delayed development and microcephaly and died at five years of age [20]. Two patients with a milder phenotype had an InsA105 mutation [13]. This mutation was also found in compound with a missense mutation R460G in an infant with delayed developmental lactic acidosis [21]. IVS9+1G>A, a mutation at a splice site was found [11] in compound with I393T in exon 11 in a boy with recurrent hypoglycemia, impaired mental development, ataxia and elevated lactic acid, and branched chain amino acids. In a 10-week-old boy with a similar metabolic picture, two mutations, A1081G and G1123A, were found [22].

TREATMENT

Treatment has not generally been satisfactory in these patients. Most of them have died in infancy. It is important to recognize the problem because the high fat diet that is usually useful in isolated PDHC deficiency may make these patients severely acidotic [4, 8]. Restriction of protein intake may result in a decrease in the excretion of 2-oxoglutarate. This would be consistent with inhibition of human KGDHC by branched-chain ketoacids, as has been observed in pig heart KGDHC *in vitro* [23]. Treatment by restriction of protein appeared to be clinically efficacious in one patient [5], but it did not prevent a fatal outcome in this patient or another [8].

Treatment with lipoic acid was reported in one patient to produce a dramatic clearing of the abnormal organic aciduria and a reduction in lactic and pyruvic acid concentrations in blood [7]. It appeared to prevent the occurrence of episodes of acidosis and to promote consistent gains in growth and development. Doses employed were 25–50 mg/kg per day. Similar doses appeared to be of some benefit in another patient [8]. Treatment with glutamine [4] and biotin [7] have not been of benefit, nor have large doses of thiamine [4, 7]. Nevertheless, it may be prudent to administer thiamine to patients being treated with lipoic acid because lipoic acid has been reported to be toxic to thiamine-deficient animals [24]. Lipoic acid has been thought to be useful in the management of Amanita mushroom poisoning and other forms of liver disease [25]. The adult human dose was 50–150 mg i.v. every 6 hours.

Renal tubular acidosis should be managed with sodium bicarbonate or sodium citrate.

REFERENCES

- Robinson BH, Taylor J, Sherwood WG. Deficiency of dihydrolipoyl dehydrogenase (a component of pyruvate and α -ketoglutarate dehydrogenase complexes): a cause of congenital lactic acidosis in infancy. *Pediatr Res* 1977; **11**: 1198.
- Haworth JC, Perry TL, Blass JP *et al*. Lactic acidosis in three sibs due to defects in both pyruvate dehydrogenase and α -ketoglutarate dehydrogenase complexes. *Pediatrics* 1976; **58**: 564.
- Kuroda Y, Kline JJ, Sweetman L *et al*. Abnormal pyruvate and α -ketoglutarate dehydrogenase complexes in a patient with lactic acidemia. *Pediatr Res* 1979; **12**: 928.
- Robinson BH, Taylor J, Kahler SG, Kirman HN. Lactic acidemia neurologic deterioration and carbohydrate dependence in a girl with dihydrolipoyl dehydrogenase deficiency. *Eur J Pediatr* 1981; **136**: 35.
- Sakaguchi Y, Yoshino M, Aramaki S *et al*. Dihydrolipoyl dehydrogenase deficiency: a therapeutic trial with branched-chain amino acid restriction. *Eur J Pediatr* 1986; **145**: 271.
- Munnich A, Saudubray J-M, Taylor J *et al*. Congenital lactic acidosis α -ketoglutaric aciduria and variant form of maple syrup urine disease due to a single enzyme defect dihydrolipoyl dehydrogenase deficiency. *Acta Paediatr Scand* 1982; **71**: 167.
- Matalon R, Stumpf DA, Mihals K *et al*. Lipoamide dehydrogenase deficiency with primary lactic acidosis: favorable response treatment with oral lipoic acid. *J Pediatr* 1984; **104**: 65.
- Yoshida I, Sweetman L, Kulovich S *et al*. Effect of lipoic acid in patient with defective activity of pyruvate dehydrogenase and branched-chain keto acid dehydrogenase. *Pediatr Res* 1990; **27**: 75.
- Scherer SW, Otulakowski G, Robinson BH, Tsui L-C. Localization of the human dihydrolipoamide dehydrogenase gene (DLD) to 7q31-q32. *Cytogenet Cell Genet* 1991; **56**: 176.
- Sakaguchi Y, Yoshino M, Aramaki S *et al*. Dihydrolipoyl dehydrogenase deficiency: a therapeutic trial with branched-chain amino acid restriction. *Eur J Pediatr* 1986; **145**: 271.
- Grafakou O, Oexle K, van den Heuvel L *et al*. Leigh syndrome due to compound heterozygosity of dihydrolipoamide dehydrogenase gene mutations: description of the first E3 splice site mutation. *Eur J Pediatr* 2003; **162**: 714.
- Schwartz WJ, Hutchinson HT, Berg BO. Computerized tomography in subacute necrotizing encephalomyelopathy (Leigh disease). *Ann Neurol* 1981; **10**: 268.
- Elpeleg ON, Shaag A, Glustein JZ *et al*. Lipoamide dehydrogenase deficiency in Ashkenazi Jews: an insertion mutation in the mitochondrial leader sequence. *Hum Mutat* 1997; **10**: 256.
- Aptowitz I, Saada A, Faber J *et al*. Liver disease in the Ashkenazi-Jewish lipoamide dehydrogenase deficiency. *J Pediatr Gastroenterol Nutr* 1997; **24**: 599.
- Matuda S, Kitano A, Sakaguchi Y *et al*. Pyruvate dehydrogenase subcomplex with lactic acidosis and branched-chain ketoaciduria. *Clin Chim Acta* 1984; **14**: 59.
- Otulakowski G, Nyhan WL, Sweetman L, Robinson BH. Immunoextraction of lipoamide dehydrogenase from cultured skin fibroblasts in patients with combined α -ketoacid dehydrogenase deficiency. *Clin Chim Acta* 1985; **152**: 27.
- Feigenbaum AS, Robinson BH. The structure of the human dihydrolipoamide dehydrogenase gene (DLD) and its upstream elements. *Genomics* 1993; **17**: 376.
- Otulakowski G, Robinson GH. Isolation and sequence determination of cDNA clones for porcine and human lipoamide dehydrogenase: homology to other disulfide oxidoreductases. *J Biol Chem* 1987; **262**: 17313.
- Liu T-C, Kim H, Arizmendi C *et al*. Identification of two missense mutations in a dihydrolipoamide dehydrogenase-deficient patient. *Proc Natl Acad Sci USA* 1993; **90**: 5186.
- Shaag A, Saada A, Berger I *et al*. Molecular basis of lipoamide dehydrogenase deficiency in Ashkenazi Jews. *Am J Med Genet* 1999; **82**: 177.
- Hong YS, Kerr DS, Craigen WJ *et al*. Identification of two mutations in a compound heterozygous child with dihydrolipoamide dehydrogenase deficiency. *Hum Mol Genet* 1996; **5**: 1925.

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22. Cerna L, Wenchich L, Hansiková H *et al.* Novel mutations in a boy with dihydrolipoamide dehydrogenase deficiency. *Med Sci Monit* 2001; **7**: 1319.
 23. Kanzaki T, Hayakawa T, Hamada M *et al.* Mammalian α -keto acid dehydrogenase complexes. IV. Substrate specificities and kinetic properties of the pig heart pyruvate and 2-oxoglutarate dehydrogenase complexes. *J Biol Chem* 1969; **244**: 118.
 24. Gal EM, Razevska DE. Studies on the *in vivo* metabolism of lipoic acid. I. The fate of DL-lipoic acid- S^{35} in normal and thiamine deficient rats. *Arch Biochem Biophys* 1960; **8**: 253.
 25. Michel DH. Amanita mushroom poisoning. Defective activity of pyruvate dehydrogenase. *Ann Rev Med* 1980; **31**: 51.

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)

Introduction	374	Treatment	379
Clinical abnormalities	374	References	379
Genetics and pathogenesis	378		

MAJOR PHENOTYPIC EXPRESSION

Mitochondrial myopathy, shortness of stature, stroke-like episodes, seizures, encephalopathy progressive to dementia, migraine, diabetes mellitus, lactic acidemia, ragged red muscle fibers, and mutations in the mitochondrial tRNA leucine gene.

INTRODUCTION

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome was first defined as such by Pavlakis and colleagues [1] in 1984, although patients have doubtless been reported earlier. Among the mitochondrial myopathies, this is one of the more common [2].

The typical clinical presentation includes all of the features that make up the name of the syndrome, but there is enormous variability. Some affected individuals have only diabetes, or only migraine. Others have only hearing loss, or hearing loss and diabetes [3]. The disease is inherited in a maternal pattern, and the gene is on the mitochondrial genome (Figure 51.1). Most of the patients have had one of two point mutations in the mitochondrial gene for the leucine (UUR) tRNA (A3243G and T3271C) (Figure 51.2) [4–7].

CLINICAL ABNORMALITIES

There is a considerable variety of expression consistent with the varying heteroplasmy of mitochondrial inheritance. The typical picture is of normal development followed by a severe, progressive encephalomyopathy. Onset may be myopathic with exercise intolerance or weakness (Figure 51.3). Many patients have shortness of stature and this may

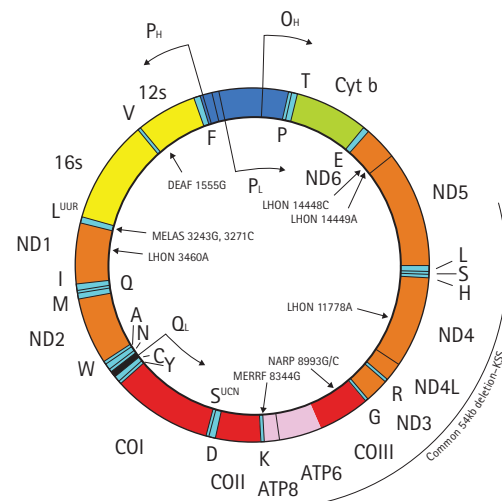


Figure 51.1 The circular DNA of the human mitochondrial genome. Shown are the sites of the mitochondrial genes, as well as the sites for the most common mutations, including the A3243G and T3271C mutations associated with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome.

be the first manifestation of disease (Figure 51.4). One of our patients had been treated unsuccessfully with human growth hormone by a pediatric endocrinologist; this has also been reported by others. In many patients, the onset of symptoms is with the first stroke-like episode, usually between four and 15 years [1, 4, 8–14]. Less commonly, the

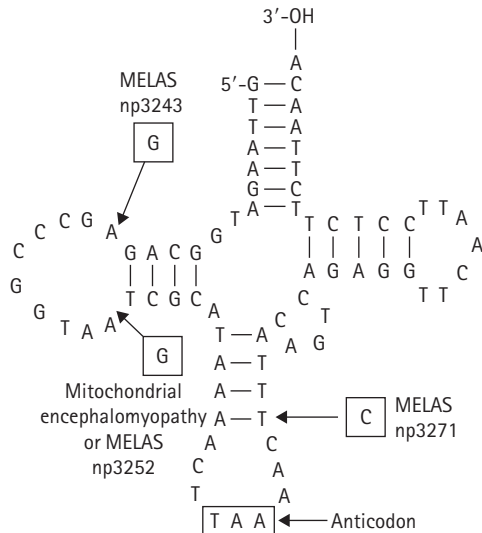


Figure 51.2 The tRNA for leucine, the site of the defect in the mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome. In addition to the point mutation at npA3243G, the common mutation in MELAS, and npT3271C and npA3252G the other MELAS mutations, there are a number of other known mutations in the tRNA leucine which cause mitochondrial diseases. These include npT3250C (mitochondrial myopathy), npA3751G (chronic progressive external ophthalmoplegia (CPEO) proximal weakness, sudden death); npA3260G (adult onset hypertrophic cardiomyopathy and myopathy), npA3302G (mitochondrial myopathy), and npC3303T (adult onset hypertrophic cardiomyopathy and myopathy).



Figure 51.3 KS: A boy with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) illustrating his lordotic, myopathic posture. He presented at four years of age with weakness and exercise intolerance. He also had insulin-dependent diabetes mellitus. Blood concentration of creatine phosphokinase (CPK) was 462 IU/L. Plasma lactate was 93.1 mg/dL. (This illustration was kindly provided by Dr Richard Haas of University of California, San Diego.)

onset of disease may be in infancy [8], often with delayed developmental milestones or learning disability.

The myopathy may be present before the first stroke. At one extreme is a floppy infant at four months of age [8]. More commonly, there is exercise intolerance, easy fatigability, or frank weakness. Patients may have difficulty going up stairs. Myopathy may be progressive. Proximal muscles tend to be more involved than the distal [8]. Musculature is generally thin. The facial appearance may be myopathic [15]. The creatine phosphokinase activity in the blood may be elevated [13, 16]. Some patients have been diagnosed as having polymyositis [11]. The electromyogram (EMG) may demonstrate a myopathic pattern.

The stroke-like episode is the hallmark feature of this syndrome. At the same time, these episodes may occur in only a few members of a pedigree, in which a much larger number has the same mutation [15, 16]. In one series of four families [16], stroke-like episodes occurred only in the probands. Two of the affected mothers were clinically entirely normal. In other pedigrees, no member may have had this defining manifestation. The episode may initially be manifest by vomiting and headache, convulsions, or visual abnormalities [8]. Less commonly, there may be

numbness, hemiplegia, or aphasia. There may be recurrent episodes of headache or vomiting lasting a few hours to several days. The episode may be followed by transient hemiplegia or hemianopia lasting a few hours to several weeks. Computed tomography (CT) or magnetic resonance imaging (MRI) scan of the brain following such an episode reveals lucency consistent with infarction (Figures 51.5 and 51.6) [17]. This picture may resolve over hours or days, but later there may be cerebral atrophy and calcifications, especially in the basal ganglia (Figure 51.6) [17–24].

Infarcts are most common in the posterior temporal, parietal, or occipital lobes, but histologic examination may reveal clear-cut infarcts widely scattered in the cerebrum, cerebellum, or basal ganglia [18, 20, 25, 26]. Thus, these episodes are in fact strokes. The term 'stroke-like' may be appropriate in that no vascular changes of inflammation or atherosclerosis are found in the brain. We have tended to refer to this type of lesion as metabolic stroke in other diseases, such as propionic acidemia (Chapter 2) or methylmalonic acidemia (Chapter 3). In MELAS, mitochondrial angiopathy is evident in contrast enhancement in affected areas [21, 27–29] and even in the skin as purpuric lesions.

The migraine or migraine-like headaches seen in these



Figure 51.4 NF: A boy with mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS) who had strokes on three occasions and had become demented. Stature was very short. (This illustration was kindly provided by Dr Richard Haas of University of California, San Diego.)

patients may reflect the same process. Headache may be hemispheric. In pedigrees of patients with classic MELAS, there are many members whose only manifestation is migraine (Figure 51.7) [8, 15]. Developmental delay, learning disability [8], or attention deficit disorder [15], is mainly found in patients prior to the development of the first stroke. This was the history of the patient

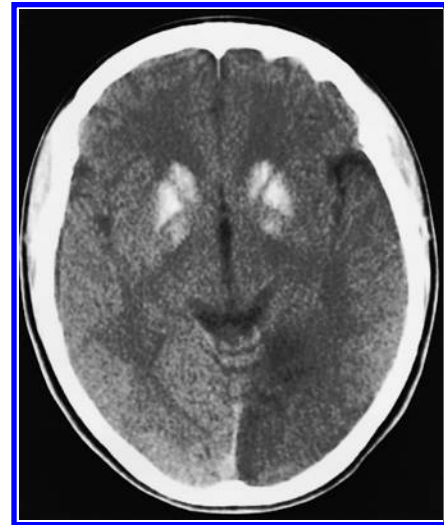


Figure 51.5 Computed tomography scan of the brain of MR, a boy with the A3243G mutation, illustrating the posterior infarct and the extensive calcifications in the basal ganglia, including the caudate, putamen and globus pallidus. (This illustration was kindly provided by Dr Richard Haas of University of California, San Diego.)

illustrated in Figure 51.4 who did not have his first stroke until the age of eight, but had been in a special education program for years. On the other hand, some patients with considerable myopathy and/or other symptomatology may be intellectually normal (Figure 51.3). The encephalopathy, when it develops, may be progressive to dementia (Figure 51.4). The patient may be apathetic and cachectic [18].

Additional neurologic features include ataxia, tremor, dystonia, visual disturbances, and cortical blindness. Some have had myoclonus. Convulsive seizures may be focal or generalized tonic-clonic, but may also be myoclonic [7].

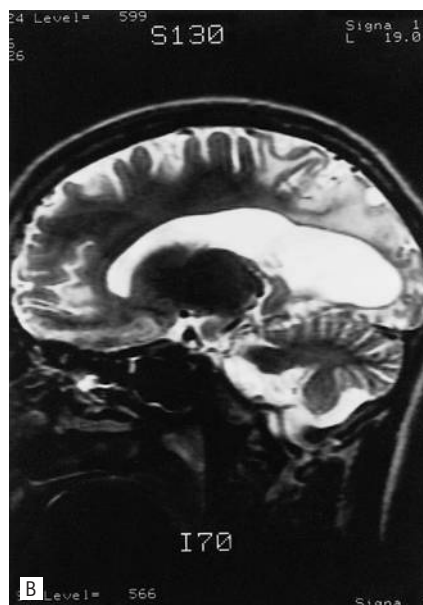
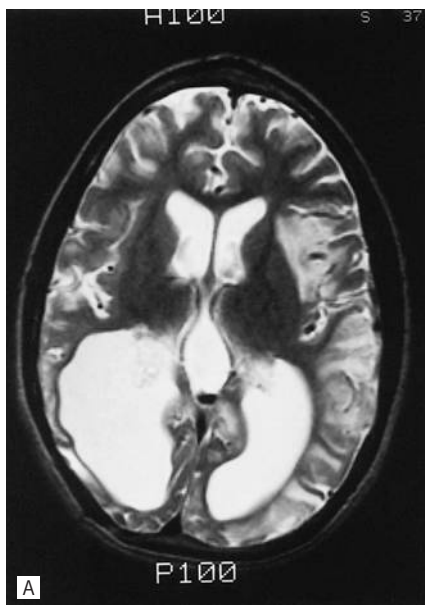


Figure 51.6 Magnetic resonance image of the brain of NF, illustrating widespread cortical atrophy, residual of a right parieto-occipital infarct with ventriculomegaly and increased T₂ signal representing preinfarction state in left temporoparieto-occipital cortex. (This illustration was kindly provided by Dr Richard Haas of University of California, San Diego.)

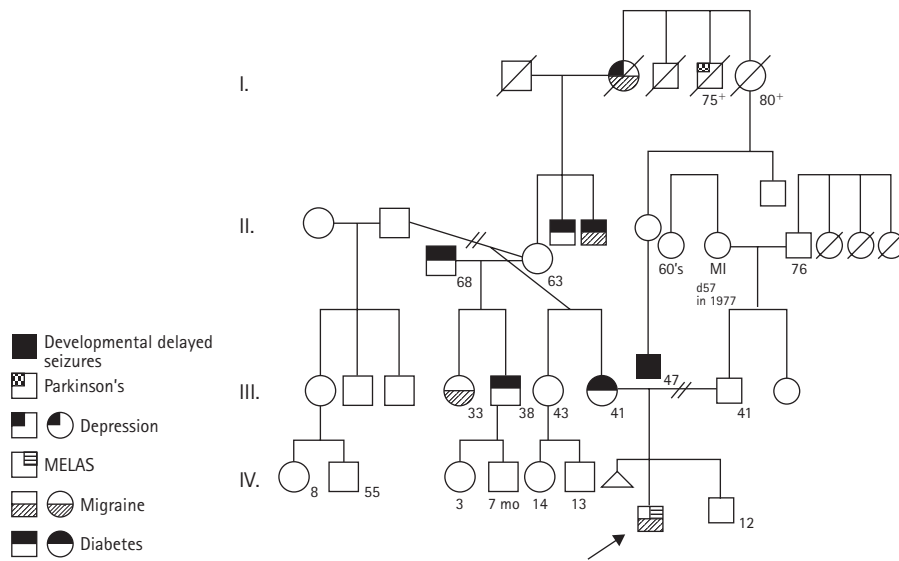


Figure 51.7 Pedigree of the family of NF, illustrating the occurrence of diabetes, migraine, seizures, and other problems. Analysis of the blood revealed the npA3243G mutation.

The electroencephalogram (EEG) is usually abnormal and there are usually epileptiform spike discharges.

Some patients have had ophthalmoplegia or ptosis [11]. Others have had pigmentary degeneration of the retina [30] like those with the neurodegeneration, ataxia, and retinitis pigmentosa (NARP) mutation ([Chapter 53](#)). Patients have been referred to as having the Kearns-Shy syndrome [11]. Others have presented with the picture of Leigh syndrome ([Chapter 46](#)), in which patients have recurrent attacks of neurologic regression, pyramidal and extrapyramidal signs, brainstem abnormalities, and leukodystrophy [31, 32].

An interesting consequence of the MELAS mutation is the occurrence of diabetes mellitus ([Figure 51.7](#)) [30]. This appears to be the most common manifestation of MELAS. It is usually type II diabetes [33], but the boy shown in [Figure 51.3](#) had insulin-dependent diabetes mellitus.

Sensorineural hearing loss is another common manifestation and it may be seen in individuals with or without diabetes and no other manifestations of disease [3]. It may also be seen in patients with the classic syndrome. Deafness has been reported in about 25 percent of patients [8]. The disease is a major cause of aminoglycoside-induced hearing loss [34]. This provides an argument for screening for the MELAS mutation in patients with antibiotic-induced deafness, in order to test affected relatives and avoid aminoglycosides in them.

Cardiomyopathy is a less common feature, but may be found in about 10 percent of patients. It is usually hypertrophic cardiomyopathy, but it may be dilated [35]. Patients with the MELAS mutations have been found to have MELAS and cardiomyopathy, but others have had isolated cardiomyopathy and no neurologic disease. There may be conduction abnormalities (for instance, Wolff-Parkinson-White syndrome [18]) and often an abnormal electrocardiogram [36]. Huge accumulation of mitochondria has been observed in myocardial fibers [18].

Renal involvement may take the form of renal tubular

acidosis, and there may be a typical renal Fanconi syndrome [37]. One patient developed a nephrotic syndrome and had focal glomerulosclerosis [16]. A variety of other organs has been involved in individual patients. One had pancreatitis following valproate administration [15]. Others have had peripheral neuropathy with or without rhabdomyolysis [38, 39]. One had ischemic colitis [40]. Pigmentary abnormalities of the skin have been reported [37].

The histologic signature of the MELAS syndrome is the appearance of ragged red fibers in the muscle ([Figure 51.8](#)) [1, 12, 13, 36]. These are best seen in the trichrome stain. In H&E, there may be variation in fiber size and increase in connective tissue. Staining with periodic acid Schiff (PAS), NADH tetrazolium reductase or for succinic dehydrogenase may show increased subsarcolemmal activity. Electron microscopy reveals an increase in number and size of mito-

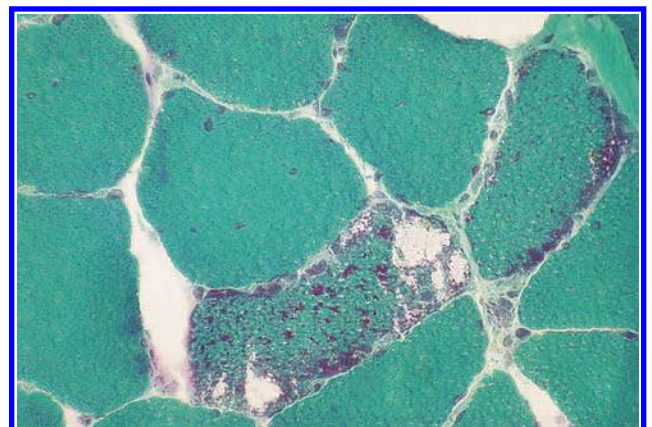


Figure 51.8 Ragged red fibers of the muscle of a patient with mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS). (This illustration was kindly provided by Dr Richard Haas of University of California, San Diego.)

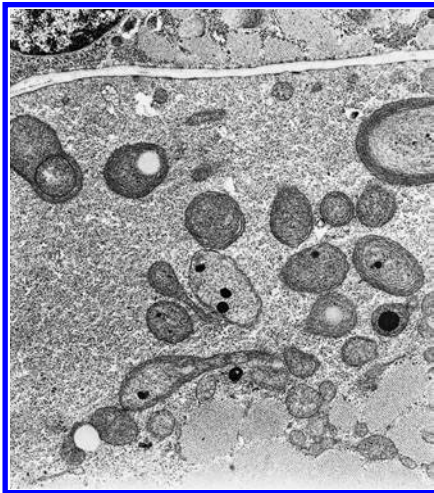


Figure 51.9 Electronmicroscopy of the muscle of the mother of KS. She had diabetes, but no symptoms of myopathy. Illustrated are many pleomorphic mitochondria, abnormal concentric lamellar cristae and electron-dense bodies. There is also glycogen accumulation. (This illustration was kindly provided by Dr Richard Haas of University of California, San Diego.)

chondria (Figure 51.9), some with paracrystalline inclusion bodies [13, 36].

The lactic acidosis is an important feature of this disorder. It does not usually lead to systemic acidosis and it may even be absent in patients with impressive involvement of the central nervous system. The levels may be elevated in cerebrospinal fluid (CSF) and normal in blood [32]. The patient in Figure 51.4 had repeated determinations of lactate in the blood in the normal range (20 mg/dL); his CSF lactate was 56.3 mg/dL. The CSF concentration of protein may be mildly elevated.

GENETICS AND PATHOGENESIS

The MELAS syndrome is the result of mutation in mitochondrial genes for tRNA [41]. The most common is A-to-G transition at position 3243 of the tRNA^{Leu}(UUR) (Figure 51.1) [4, 5]. Approximately 80 percent of affected individuals have this mutation in the dihydrouridine loop of the gene [8, 16, 42–44]. The other common mutation, occurring in about 8.5 percent of individuals, is also in the tRNA^{Leu}(UUR) at 3271 in the anticodon, where there is a T-to-C transversion [7]. The G-to-A transversion at 3252 of the same gene has been reported in mitochondrial encephalopathy [45]. Another mutation in the dihydrouridine loop at nucleotide 3250 is a T-to-C transition [42]. Another mutation in this gene is an A-to-T change at position 3256 [46]. A 5814 G in the tRNA^{Cys} gene was reported in a patient with cardiomyopathy and myopathy [35].

A quite distinct mutation, an A-to-G transition at

nucleotide 11084 in the ND4 gene for the subunit of complex I of the respiratory chain, was reported by Lertrit *et al.* [47] in a Caucasian patient. This same mutation was later reported by Sakuta and colleagues [48] in 10–14 percent of Japanese studied, both patients with mitochondrial myopathy and normal controls, suggesting that it might be a polymorphism. On the other hand, this mutation was not found in 109 normal or patient Caucasians nor in American Blacks, nor in a considerable number of patients with other mitochondrial diseases. Thus, the issue on this transition is unresolved. A heteroplasmic mutation in the ND6 gene was found in a seven-year-old whose onset was with vomiting and ketoacidosis, and who went on to develop ataxia, myoclonic seizures, and multiple infarctions [49]. The mutation was c.14453G>A. Mutations were also reported in the *MTTA* and *MTNDS* genes [49]. A c.4332G>A mutation was found in a 47-year-old man with sensorineural deafness who presented with a stroke [50]. A heteroplasmic mutation in the *MTTH* gene was found in a patient with sudden migraine followed by hemiparesis [51]. A large (10.5 kb) deletion was reported in a MELAS patient with a renal Fanconi syndrome [37]. Late onset at 50 years was observed in a patient with the common c.3243A>G mutations whose onset with headaches and seizures was associated with bitemporal lesions [52]. In 23 patients with the common mutation, 77 percent had abnormal peripheral nerve conduction [53]. Autonomic symptoms, such as gastrointestinal symptoms, orthostatic dizziness, and cold or discolored hands and feet were reported [54] in 80 percent of 35 patients with the common mutation [54]. In another approach to relate

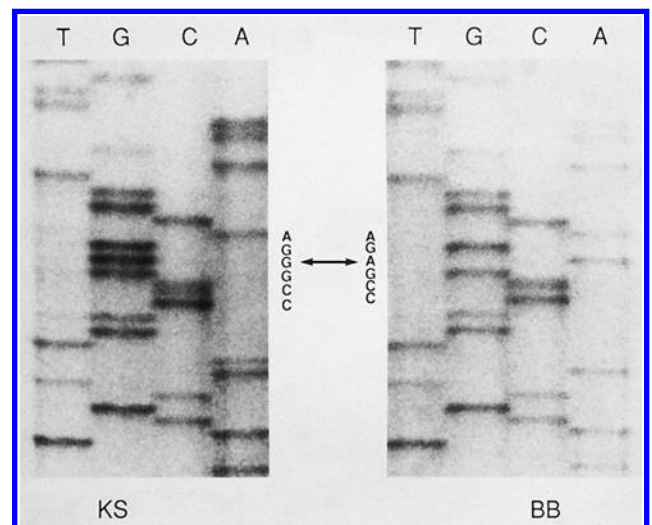


Figure 51.10 Sequencing gel of the mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS) region of the leucine tRNA of muscle. The npA3243G mutation was in KS; BB was a normal control. (This illustration was kindly provided by Dr Richard Haas of University of California, San Diego.)

clinical features with mutation, deafness was the most common feature in 52 adults with the common mutation [55].

The common mutation creates a new site for the restriction endonuclease *Hae*III leading to a 169-bp fragment in controls after electrophoresis and fragments of 97 and 72 bp in patients with MELAS [43]. Sequencing (Figure 51.10) reveals the G in MELAS where there is an A in control. Varying heteroplasmy among affected individuals appears to reflect variable segregation in the ovum. On the other hand, study of the proportion of mutant DNA in various tissues obtained from a woman and her two daughters revealed similar proportions in tissues derived from ectodermal, endodermal, and mesodermal germ layers, indicating little mitotic segregation after early embryogenesis [56]. The issue of heteroplasmy, which can vary from tissue to tissue making detection difficult has been addressed in MELAS A3243G by the design of peptide nucleic acids which bond to the wild-type mtDNA at 3243 preventing polymerase chain reaction (PCR) amplification and making the mutant the dominant product [57].

Mutations in the tRNA for leucine might be expected to have an important effect on translation and hence protein synthesis in mitochondria. This has been demonstrated in studies of cybrids [25] by fusing human cell lines lacking mitochondrial DNA with exogenous mitochondria containing 0 to 100 percent of the common 3243 mutant DNA. Cybrids containing more than 95 percent mutant DNA had decreased rates of synthesis and steady-state levels of mitochondrial proteins leading to respiratory chain deficiency.

Patients with the MELAS syndrome have been found to have marked deficiency in the activity of complex I of the respiratory chain [12]. In mitochondria from muscle, rotenone-sensitive NADH-cytochrome reductase activity was 0–27 percent of control value, and immunochemical study revealed a general decrease in complex I subunits. In a patient with the T-to-C 3250 mutation, complex I activity in muscle was 6 percent of control and that of complex IV was 47 percent of control [58]. The production of CO₂ from labeled pyruvate, malate, and 2-oxoglutarate was in each case reduced [36]. In a study of four patients with the 3243 mutation, the activity of complexes I and IV were reduced in muscle and other tissues, but there was no correlation between the proportion of mutant DNA in a tissue and the activity of the respiratory chain complexes [44].

The pathophysiology of stroke in this disease is considered to reflect a mitochondrial role in endothelial or other vascular dysfunction [59].

TREATMENT

A variety of supportive measures is helpful in this disorder, as in other mitochondrial diseases. Riboflavin therapy has been reported to be of benefit in a patient with complex

I deficiency and the T-to-C 3250 mutation [58]. A dose of 20 mg twice a day was employed in a two-year-old patient with myopathy who could not ascend stairs and was reluctant to walk. Improvement in muscle strength occurred, and there was no further deterioration over three years of observation.

Coenzyme Q has been helpful in a number of patients [14]. Some amelioration of muscle weakness has been observed, as well as some decrease in plasma levels of lactate. CSF lactate did not improve. Doses of 30–90 mg/day were reported [14]. In MELAS, doses as high as 300 mg/day have been stated to be required for optimal effects [13, 14].

Experience with dichloroacetic acid (Chapter 46) is accumulating; it is clear that levels of lactate are lowered in both plasma and CSF. MELAS has been one of the disorders that responded temporarily favorably to this agent.

Patients with myoclonus may be effectively treated with lamotrigine [60].

Arginine has been used intravenously in the acute stroke situation in a dose of 500 mg/kg over 90 minutes. A long-term oral maintenance dose was 0.3 g/kg. Plasma concentrations of arginine and citrulline are low in patients with MELAS syndrome. Both are nitric oxide precursors, and it has been proposed that citrulline would be a better source than arginine which requires a transporter to enter the cell. A study of arginine flux and nitric oxide production is under way in patients treated with either arginine or citrulline [61].

REFERENCES

1. Pavlakis SG, Phillips PC, DiMauro S *et al.* Mitochondrial myopathy encephalopathy lactic acidosis and stroke-like episodes: a distinctive clinical syndrome. *Ann Neurol* 1984; **16**: 481.
2. Hirano M, Ricci E, Koenigsberger MR *et al.* MELAS: an original case and clinical criteria for diagnosis. *Neuromusc Disord* 1992; **2**: 125.
3. Fischel-Ghodsian N. Mitochondrial mutations and hearing loss: paradigm for mitochondrial genetics. *Am J Hum Genet* 1998; **62**: 15.
4. Goto Y-I, Nonaka I, Horai S. A mutation in the tRNA^{Leu}(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature* 1990; **348**: 651.
5. Kobayashi Y, Momoi MY, Tominaga K *et al.* A point mutation in the mitochondrial tRNA^{Leu}(UUR) gene in MELAS (mitochondrial myopathy encephalopathy lactic acidosis and stroke-like episodes). *Biochem Biophys Res Commun* 1990; **173**: 816.
6. Goto Y, Nonaka I, Horai S. An alternative mutation in the mitochondrial tRNA^{Leu}(UUR) gene associated with MELAS. *Am J Hum Genet* 1991; **49**(Suppl.): 190.
7. Goto Y-I, Nonaka I, Horai S. A new mutation in the tRNA^{Leu}(UUR) gene associated with mitochondrial myopathy lactic acidosis and stroke-like episodes. *Biochim Biophys Acta* 1991; **1097**: 238.

8. Kobayashi M, Nonaka I. Mitochondrial myopathy encephalopathy lactic acidosis and stroke-like episodes (MELAS): a correlative study of the clinical features and mitochondrial DNA mutation. *Neurology* 1992; **42**: 545.
9. Montagna P, Gallassi R, Medori R *et al*. MELAS syndrome: characteristic migrainous and epileptic features and maternal transmission. *Neurology* 1988; **38**: 751.
10. Ciafaloni E, Ricci E, Shanske S *et al*. MELAS: clinical features biochemistry and molecular genetics. *Ann Neurol* 1992; **31**: 391.
11. Yoda S, Terauchi A, Kitahara F, Akabane T. Neurologic deterioration with progressive CT changes in a child with Kearns-Shy syndrome. *Brain Dev* 1984; **6**: 323.
12. De Quick M, Lammens M, Dom R, Carton H. MELAS: a family with paternal inheritance. *Ann Neurol* 1991; **29**: 456.
13. Goda S, Hamada T, Ishimoto S *et al*. Clinical improvement after administration of coenzyme Q10 in a patient with mitochondrial encephalomyopathy. *J Neurol* 1987; **234**: 62.
14. Yamamoto M, Sato T, Anno M *et al*. Mitochondrial myopathy encephalomyopathy lactic acidosis and stroke-like episodes with recurrent abdominal symptoms and coenzyme Q10 administration. *J Neurol Neurosurg Psychiatry* 1987; **50**: 1475.
15. Dougherty FE, Ernst SG, Aprille JR. Familial recurrence of atypical symptoms in an extended pedigree with the syndrome of mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes (MELAS). *J Pediatr* 1994; **125**: 758.
16. Inui K, Fukushima H, Tsukamoto H *et al*. Mitochondrial encephalomyopathies with the mutation of the mitochondrial tRNA^{Leu}(UUR) gene. *J Pediatr* 1992; **120**: 62.
17. Kobayashi M, Morishita H, Sugiyama N *et al*. Mitochondrial myopathy encephalopathy lactic acidosis and stroke-like episodes syndrome and NADH-CoQ reductase deficiency. *J Inherit Metab Dis* 1986; **9**: 301.
18. Bogousslavsky J, Perentes E, Deruaz JP, Regli F. Mitochondrial myopathy and cardiomyopathy with neurodegenerative features and multiple brain infarcts. *J Neurol Sci* 1982; **55**: 351.
19. Shapira Y, Cederbaum SD, Cancilla PA *et al*. Familial poliodystrophy mitochondrial myopathy and lactate academia. *Neurology* 1975; **25**: 614.
20. Kuriyama M, Umezaki H, Fukuda Y *et al*. Mitochondrial encephalomyopathy with lactate-pyruvate elevation and brain infarctions. *Neurology* 1984; **34**: 72.
21. Hasuo K, Tamura S, Yasumori K *et al*. Computed tomography and angiography in MELAS (mitochondrial myopathy encephalopathy lactic acidosis and stroke-like episodes); report of 3 cases. *Neuroradiology* 1987; **29**: 393.
22. Matthews PM, Tampieri D, Berkovic SF *et al*. Magnetic resonance imaging shows specific abnormalities in the MELAS syndrome. *Neurology* 1991; **41**: 1043.
23. Abe K, Inui T, Hirono N *et al*. Fluctuating MR images with mitochondrial encephalopathy lactic acidosis stroke-like syndrome (MELAS). *Neuroradiology* 1990; **32**: 77.
24. Rosen L, Phillips S, Enzmann D. Magnetic resonance imaging in MELAS syndrome. *Neuroradiology* 1990; **32**: 168.
25. Ohama E, Ohara S, Ikuta F *et al*. Mitochondrial angiography in cerebral blood vessels of mitochondrial encephalomyopathy. *J Dermatol* 1991; **18**: 295.
26. Fujii T, Okuno T, Ito M *et al*. CT MRI and autopsy findings in brain of a patient with MELAS. *Pediatr Neurol* 1990; **6**: 253.
27. Allard JC, Tilak S, Carter AP. CT and MR of MELAS syndrome. *Am J Neuroradiol* 1988; **9**: 1234.
28. Tokunaga M, Mita S, Sakuta R *et al*. Increased mitochondrial DNA in blood vessels and ragged-red fibers in mitochondrial myopathy encephalopathy lactic acidosis and stroke-like episodes (MELAS). *Ann Neurol* 1993; **33**: 275.
29. Ohama E, Ohara S, Ikuta F *et al*. Mitochondrial angiopathy in cerebral blood vessels of mitochondrial encephalomyopathy. *Acta Neuropathol* 1987; **74**: 226.
30. King MP, Koga Y, Davidson M, Schon EA. Defects in mitochondrial protein synthesis and respiratory chain activity segregate with the tRNA^{Leu}(UUR) mutation associated with mitochondrial myopathy encephalopathy lactic acidosis and stroke-like episodes. *Mol Cell Biol* 1992; **12**: 480.
31. Dahl H-HM. Getting to the nucleus of mitochondrial disorders: identification of respiratory chain-enzyme genes causing Leigh syndrome. *Am J Hum Genet* 1998; **63**: 1594.
32. Rahman S, Blok R, Dahl H-HM *et al*. Leigh syndrome: clinical features and biochemical and DNA abnormalities. *Ann Neurol* 1996; **39**: 343.
33. Van den Ouweland JMW, Lemkes HHPJ, Ruitenbeek W *et al*. Mutation in mitochondrial tRNA^{Leu}(UUR) gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. *Nat Genet* 1992; **1**: 368.
34. Prezant TR, Agapian JV, Bohlman MC *et al*. Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nat Genet* 1993; **4**: 289.
35. Karadimas C, Tanji K, Geremek M *et al*. A5814G mutation in mitochondrial DNA can cause mitochondrial myopathy and cardiomyopathy. *J Child Neurol* 2001; **16**: 531.
36. Kobayashi M, Morishita H, Sugiyama N *et al*. Two cases of NADH-coenzyme Q reductase deficiency: relationship to MELAS syndrome. *J Pediatr* 1987; **110**: 223.
37. Campos Y, Garcia-Silva T, Barrionuevo CR *et al*. Mitochondrial DNA deletion in a patient with mitochondrial myopathy lactic acidosis and stroke-like episodes (MELAS) and Fanconi's syndrome. *Pediatr Neurol* 1995; **13**: 69.
38. Hara H, Wakayama Y, Kouno Y *et al*. Acute peripheral neuropathy rhabdomyolysis and severe lactic acidosis associated with 3243 A to G mitochondrial DNA mutation. *J Neurol Neurosurg Psychiatry* 1994; **57**: 1545 (letter).
39. Rusanen H, Majamaa K, Tolonen U *et al*. Demyelinating polyneuropathy in a patient with the tRNA^{Leu}(UUR) mutation at base pair 3243 of the mitochondrial DNA. *Neurology* 1995; **45**: 1188.
40. Hess J, Burkhard P, Morris M *et al*. Ischaemic colitis due to mitochondrial cytopathy. *Lancet* 1995; **346**: 189 (letter).
41. Enter C, Muller HJ, Zierz S *et al*. A specific point mutation in the mitochondrial genome of Caucasians with MELAS. *Hum Genet* 1991; **88**: 233.
42. Goto Y, Tojo M, Tohyama J *et al*. A novel point mutation in the mitochondrial tRNA^{Leu}(UUR) gene in a family with mitochondrial myopathy. *Ann Neurol* 1992; **31**: 672.
43. Moraes CT, Ricci E, Bonilla E *et al*. The mitochondrial tRNA^{Leu}(UUR) mutation in mitochondrial encephalomyopathy

- lactic acidosis and stroke-like episodes (MELAS): genetic biochemical and morphological correlations in skeletal muscle. *Am J Hum Genet* 1992; **50**: 934.
44. Obermaier-Kusser B, Paetzke-Brunner I, Enter C *et al*. Respiratory chain activity in tissues from patients (MELAS) with a point mutation of the mitochondrial genome [tRNA(Leu(UUR))]. *FEBS Lett* 1991; **286**: 67.
 45. Morten KJ, Cooper JM, Brown GK *et al*. A new point mutation associated with mitochondrial encephalomyopathy. *Hum Mol Genet* 1993; **2**: 2081.
 46. Sato W, Hayasaka K, Shoji Y *et al*. A mitochondrial tRNA^{Leu(UUR)} mutation at 3256 associated with mitochondrial myopathy encephalopathy lactic acidosis and stroke-like symptoms (MELAS). *Biochem Mol Biol Int* 1994; **33**: 1055.
 47. Lertrit P, Noer AS, Jean-Francois MJB *et al*. A new disease-related mutation for mitochondrial encephalopathy lactic acidosis and stroke-like episodes (MELAS) syndrome affects the ND4 subunit of the respiratory complex. *Am J Hum Genet* 1992; **51**: 457.
 48. Sakuta R, Goto Y, Nonaka I, Horai S. An A-to-G transition at nucleotide pair 11084 in the ND4 gene may be an mtDNA polymorphism. *Am J Hum Genet* 1993; **53**: 964 (letter).
 49. Ravn K, Wilbrand F, Hansen FJ *et al*. An mtDNA mutation, 14453G→A, in the NADH dehydrogenase subunit 6 associated with severe MELAS syndrome. *Eur J Hum Genet* 2001; **9**: 805.
 50. Bataillard M, Chatzoglou E, Rumbach L *et al*. A typical MELAS syndrome associated with a new mitochondrial tRNA glutamine point mutation. *Neurology* 2001; **13**: 56.
 51. Melone MA, Tessa A, Petrini S *et al*. Revelation of a new mitochondrial DNA mutation (G12147A) in a MELAS/MERFF phenotype. *Arch Neurol* 2004; **61**: 269.
 52. Kisanuki YY, Gruis KL, Smith TL. Late-onset mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes with bimodal lesions. *Arch Neurol* 2006; **63**: 1200.
 53. Kaufmann P, Pascual JM, Anziska Y *et al*. Nerve conduction abnormalities in patients with MELAS and the A3243G mutation. *Arch Neurol* 2008; **63**: 746.
 54. Parsons T, Weimer L, Engelsta K *et al*. Autonomic symptoms in carriers of the m.3243A.G mitochondrial DNA mutation. *Arch Neurol* 2010; **67**: 976.
 55. Deschauer M, Müller T, Wieser T *et al*. Hearing impairment is common in various phenotypes of the mitochondrial DNA A3243G mutation. *J Am Med Assoc* 2002; **287**: 698.
 56. McMillan C, Shoubridge EA. Variable distribution of mutant mitochondrial DNAs (tRNA^{Leu(3242)}) in tissues of symptomatic relatives with MELAS: the role of mitotic segregation. *Neurology* 1993; **43**: 82P (Abstr.).
 57. Hancock DK, Schwarz FP, Song F *et al*. Design and use of a peptide nucleic acid for detection of the heteroplasmic low-frequency mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes (MELAS) mutation in human mitochondrial DNA. *Clin Chem* 2002; **48**: 2155.
 58. Ogle RF, Christodoulou J, Fagan E *et al*. Mitochondrial myopathy with tRNA^{Leu(UUR)} mutation and complex I deficiency responsive to riboflavin. *J Pediatr* 1997; **130**: 138.
 59. Testai FD, Gorelick PB. Inherited metabolic disorders and stroke part 1: Fabry disease and mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. *Arch Neurol* 2010; **67**: 19.
 60. Costello DJ, Sims KB. Efficacy of lamotrigine in disabling myoclonus in a patient with an mtDNA A3243G mutation. *Neurology* 2009; **72**: 1279.
 61. El-Hattab A, Craigen W, Wong L-J. Arginine flux and nitric oxide production in subjects with MELAS syndrome and the effect of arginine and citrulline supplementation: study design. *Mol Genet Metab* 2010; **99**: 187 (Abstr.).

Myoclonic epilepsy with ragged red fibers (MERRF) was first reported in 1973 by Tsairis and colleagues [4], but current recognition of the disease as a distinct entity was focused by the report of Fukuhara and his associates in 1980 of two patients with myoclonic epilepsy and ragged red fibers [5]. By 1988, 25 examples of the MERRF disease were reviewed by the Columbia group [6]. In the same year, Wallace and colleagues reported evidence that the disease was maternally inherited and qualified as a disease of mitochondrial DNA [7]. This group reported the point mutation in the gene for the lysine tRNA in 1990 (Figure 52.1) [8]. The missense mutation A8344G has been found in approximately 80 percent of patients [8, 9].

CLINICAL ABNORMALITIES

Myoclonic seizures are characteristic features of the disease [10–12]. They have been reported along with ataxia in the absence of ragged red fibers on muscle biopsy in patients with the documented mutation [13]. However, there is considerable phenotypic variability. In a single family with clear maternal inheritance, the clinical picture ranged all the way from an 18-year-old female who had myoclonus, ataxia, deafness, spasticity, and dementia to asymptomatic status with ragged red fiber histology in two members of the family [13]. This is consistent with variable heteroplasmy. The classic picture is of a progressive myoclonic epilepsy, mitochondrial myopathy with ragged red fibers, and slowly progressive dementia (Figures 52.2 and 52.3) [7, 10–23]. Patients may also have akinetic seizures [10] or generalized grand mal seizures [24]. Myoclonic jerks may be virtually continuous and may dominate the clinical picture [10]. The onset of symptoms may be in late childhood or in adulthood [5, 7, 10–12, 14–16, 25, 26]. Truncal and limb ataxia are present, and speech may be scanning. There may be spastic paraparesis, exaggerated deep tendon reflexes in the legs and extensor plantar responses [10]. Migraine was observed in a 20-year-old woman, who had had panic attacks at 11 years, and developed exercise intolerance,



Figure 52.2 A 12-year-old boy with the MERRF disease. He could walk, but the muscle weakness is indicated by his stooping posture. His pedigree is that of Family A of which he was the first proband in the paper of Larsson *et al.* [58]; other members of the family had lipomas of the neck. (This illustration and Figure 52.3 were kindly provided by Dr Mär Tulinus of the Department of Pediatrics, Sahlgrenska University Hospital Östra, Göteborg, Sweden.)

ataxia, hearing loss, and problems of balance [27]. Progressive loss of balance was also observed in a patient who developed seizures and myoclonus at 27 years, and sensorineural deafness at 37 years [28].

Optic atrophy is common [10]. Eye movements are visually normal, but ocular apraxia has been observed in one patient [25] and abnormal mitochondria have been observed in extraocular muscles [25], along with endomysial fibrosis [29, 30]. Paracrystalline inclusions may be absent. Hearing loss is characteristic [7, 12, 16, 17, 21, 30], but some patients have normal hearing [5, 15, 25, 31]. Stature is usually short [10, 11]. Peripheral neuropathy may be evident clinically; nerve conduction velocity is often reduced [10]. An absence of strokes has been used to distinguish this disorder from mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes (MELAS) (Chapter 51). However, a family has now been reported [32] in which the 20-year-old proband developed sudden migraine followed by left hemiparesis and homonymous hemianopia, as well as seizures. The picture was judged to be that of MELAS. At 25 years, he went on to develop myoclonus and ataxia indicating progression to MERRF. A third generation Sardinian family also had characteristics of both MELAS and MERRF [33]. An unusual finding seen in a few patients is the occurrence of multiple lipomas in the neck and trunk [24, 34].

The electroencephalogram (EEG) is characteristically abnormal. The typical pattern is of frequent bilateral



Figure 52.3 The same patient at 16 years of age. By this time, he was wheelchair-bound. He died at 18 years of age. Autopsy information was reported by Oldfors and colleagues [59]. The brain contained mutant mitochondrial DNA in 91–99 percent of tissues studied.

episodes of high voltage delta waves early in the disease and bilaterally synchronous bursts of slow spike and wave complexes later [10]. Visual evoked responses (VER) may be reduced [12]. Positron emission tomography of the brain and ^{31}P -nuclear magnetic resonance spectroscopy of brain and muscle have provided evidence of impairment of energy metabolism and mitochondrial capacity to generate adenosine triphosphate (ATP) [7, 18, 35].

Pathologic examination reveals, in addition to the characteristic myopathy with ragged red fibers (Figure 51.8), widespread neurodegeneration in the dentatorubral and pallidal systems, cerebral cortex, cerebellum, pons, and spinal cord [20, 36–39]. Staining of the muscle for cytochrome oxidase activity may show profound deficiency [10].

Blood concentrations of lactic acid are characteristically elevated, but there are exceptions, as in the case of most features of the disease, and levels are not usually very high [40]. Cerebrospinal fluid (CSF) concentrations of lactate are often higher than those of the blood. The CSF concentration of protein may be elevated, but it is usually normal [11].

GENETICS AND PATHOGENESIS

Inheritance of MERRF disease is maternal; the mutation is in the mitochondrial gene for the tRNA for lysine. The most common cause is the point mutation at nucleotide 8344 in which there is a G-to-A substitution [8, 16, 35]. This mutation accounts for 80–90 percent of patients [41–46]. In two families, another mutation, a T-to-C change at 8356, has been identified [33, 47]. A different tRNA mutation was reported [48] in a patient with manifestations called a MERRF phenotype and a mutation in the tRNA gene for leucine (MTTL), a C-to-T transition at nucleotide 3256. This patient developed tonic-clonic seizures at 28 years of age. At 45, he had limb myoclonus, mild weakness of neck muscles and deltoids, mild ataxia, and ragged red fibers in muscle. He did not have myoclonic seizures. In addition, he had hypothyroidism following thyroiditis, ptosis, ophthalmoparesis, hearing loss, diabetes mellitus, loss of central vision, optic atrophy, and retinal pigmentary degeneration. A novel mutation in the mitochondrial tRNA phenylalanine (MTTF) was found in a woman with the typical MERRF phenotype [27]. It is clear that mutations elsewhere than in the tRNA_{lysine} can produce the MERRF disease. Mutation in the tRNA histidine (MTTH) has been identified [32], as well as in the two serine tRNAs (MTS1 and MTS2) [49, 50].

Abnormalities in tRNA would be expected to lead to impairment of mitochondrial translation and protein synthesis. Consistent with this hypothesis, studies of oxidative phosphorylation in muscle have revealed reduction in the activities of complexes I and IV [7, 46], in which many of the protein components of the complexes are encoded by mitochondrial DNA [51, 52]. Patients

with MERRF have also been reported to have defects in complexes II and III [16, 18, 53]. Differences could suggest secondary effects or the substantial problems with methodology in assessing oxidative phosphorylation. On the other hand, phenotypic differences among patients tend to correlate with oxidative phosphorylation capacity of muscle [31].

Mitochondrial genetics differs from nuclear genetics in that the mitochondrial genome is inherited exclusively from the mother [54]. The mitochondrial DNA is transmitted via the cytoplasm of the egg. A cell may contain hundreds of mitochondria; during ovum formation, the number of mitochondria increases while the number of DNAs per mitochondrion decreases to one to two [55]. With growth and development, differences emerge among tissues in mitochondrial content and amounts of mitochondrial DNA. The latter is highest in brain, the organ most vulnerable to diseases of oxidative phosphorylation [56]. Cells may contain more than one sequence of mitochondrial DNA; this is referred to as heteroplasmy.

In MERRF, the rule is for heteroplasmy for the mutation, and there is enormous variation within kindred in the amounts of mutated DNA and even among tissues in a patient. In oogenesis, there is random segregation of mitochondria with and without mutation into daughter cells, accounting for the difference within a family. Random distribution during cytokinesis leads to different patterns in different tissues. Each tissue appears to have a threshold of production of mitochondrial ATP for adequate cellular function. As the percentage of abnormal mitochondrial DNA increases in different individuals, the threshold is exceeded and clinical disease results. A relatively high proportion of mutant DNA leads to clinical symptomatology [46]. Mitochondrial DNA also has a higher rate of mutation than does nuclear DNA [57]. One of the effects of ageing is an increase in the number of mutations in mitochondrial DNA. Thus, in a family with MERRF, the severity of clinical phenotype correlates with the percentage of abnormal mutant DNA and the age of the individual. Most are phenotypically normal in infancy and childhood. As age-related decrease in oxidative phosphorylation exceeds the threshold for expression in an organ, symptoms of that organ's dysfunction appear, and they become progressively more severe with age [31]. In affected individuals, the greatest percentage of mutant DNA has been found in muscle [51, 58].

For this reason, the work up of a patient thought to be a candidate for this diagnosis may require muscle biopsy. The mutation may be found by analysis of the DNA of lymphocytes or platelets, but the diagnosis cannot be excluded unless the mitochondrial DNA of muscle is analyzed. Rapidly proliferating cells, such as lymphocytes, tend to have lower proportions of mutant DNA, suggesting selection against cells with high mutant content [46].

The MERRF 8344 mutation has been shown to interfere with mitochondrial protein synthesis [51, 52]. When mutant mitochondrial DNA was greater than 85 percent,

there was impressively low synthesis of mitochondrial protein and parallel low levels of complex I and cytochrome oxidase. The effect on translation has been shown by the formation of cybrids, cells into which mitochondria were microinjected. The recipient ρ^0 cells lack mitochondrial DNA and are deficient in dihydrouridine dehydrogenase required for the synthesis of uridinemonophosphate (UMP), and thus they require uridine for growth. Microinjection of human mitochondria permits growth in the absence of uridine, but cells receiving the MERRF 8344 mutation have markedly deficient synthesis of mitochondrial DNA, while those receiving normal mitochondrial DNA synthesize mitochondrial protein well [55].

Family members at risk for maternally inherited MERRF may be tested for the mutation. Most often this is done on blood in those without symptoms. Examination of muscle may be required in those with any symptoms. Prenatal diagnosis is not generally reliable.

TREATMENT

Patients with this disease require supportive therapy aimed at the multiple systems involved. Seizures are managed with conventional anticonvulsant therapy.

Specific therapy is not yet available. Patients with disorders of oxidative phosphorylation, including MERRF, are generally treated with coenzyme Q, because of its place in the electron transport chain (Chapter 46). Doses of 4 mg/kg per day have usually been employed. Others have received riboflavin in doses of 100 mg/day.

Dichloroacetate is effective in lowering concentrations of lactic acid. Clinical improvement in MERRF patients has not been observed.

REFERENCES

- Luft R, Ikkos D, Palmieri G *et al*. A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: a correlated clinical biochemical and morphological study. *J Clin Invest* 1962; **41**: 1776.
- Engel WK, Cunningham GG. Rapid examination of muscle tissue. An improved method for fresh-frozen biopsy sections. *Neurology* 1963; **13**: 919.
- Gonatas NK, Shy GM. Childhood myopathies with abnormal mitochondria. *Excerpta Medica Int Congr Series* 1965; **100**: 606.
- Tsairis P, Engel W, Kark P. Familial myoclonic epilepsy syndrome associated with skeletal muscle mitochondrial abnormalities. *Neurology* 1973; **23**: 408.
- Fukuhara N, Tokiguchi S, Shirakawa K, Tsubaki T. Myoclonus epilepsy associated with ragged-red fibers (mitochondrial abnormalities): disease entity or a syndrome? Light- and electron-microscopic studies of two cases and review of the literature. *J Neurol Sci* 1980; **47**: 117.
- Pavakis SG, Rowland LP, De Vivo DC *et al*. Mitochondrial myopathies and encephalomyopathies. In: Plum F (ed.). *Advances in Contemporary Neurology*. New York: FA Davis, 1988: 37.
- Wallace DC, Zheng XX, Lott MT *et al*. Familial mitochondrial encephalomyopathy (MERRF): genetic pathophysiological and biochemical characterization of a mitochondrial DNA disease. *Cell* 1988; **55**: 601.
- Shoffner JM, Lott MT, Lezza AM *et al*. Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation. *Cell* 1990; **61**: 931.
- Kogelnik AM, Lott MT, Brown MD *et al*. A human mitochondrial genome database. Atlanta Center for Molecular Medicine Emory University School of Medicine. Accessed December 2004. Available from: www.mitomap.org.
- Tulinius MH, Holme E, Kristiansson B *et al*. Mitochondrial encephalomyopathies in childhood. II. Clinical manifestations and syndromes. *J Pediatr* 1991; **119**: 251.
- De Vivo D. The expanding clinical spectrum of mitochondrial diseases. *Brain Dev* 1993; **15**: 1.
- Rosing HS, Hopkins LC, Wallace DC *et al*. Maternally inherited mitochondrial myopathy and myoclonic epilepsy. *Ann Neurol* 1985; **17**: 228.
- Hammans SR, Sweeney MG, Brockington M *et al*. Mitochondrial encephalopathies: molecular genetic diagnosis from blood samples. *Lancet* 1991; **337**: 1311.
- Fitzsimmons RB, Clifton-Bligh P, Wolfenden WH. Mitochondrial myopathy and lactic acidemia with myoclonic epilepsy ataxia and hypothalamic infertility: a variant of Ramsay-Hunt syndrome. *J Neurol Neurosurg Psychiatry* 1981; **44**: 79.
- Feit H, Kirkpatrick J, Van Woert MH, Pandian G. Myoclonus ataxia and hypoventilation: response to l-5-hydroxytryptophan. *Neurology* 1983; **33**: 109.
- Morgan-Hughes JA, Hayes DJ, Clark JB *et al*. Mitochondrial encephalomyopathies: biochemical studies in two cases revealing defects in the respiratory chain. *Brain* 1982; **105**: 553.
- Holliday PL, Climie AR, Gilroy J, Mahmud MZ. Mitochondrial myopathy and encephalopathy: three cases – a deficiency of NADH-CoQ dehydrogenase? *Neurology* 1983; **33**: 1619.
- Berkovic SF, Carpenter S, Evans A *et al*. Myoclonus epilepsy and ragged-red fibres (MERRF). 1. A clinical pathological biochemical magnetic resonance spectrographic and positron emission tomographic study. *Brain* 1989; **112**: 1231.
- Lombes A, Mendell JR, Nakase H *et al*. Myoclonic epilepsy and ragged-red fibers with cytochrome oxidase deficiency: neuropathology biochemistry and molecular genetics. *Ann Neurol* 1989; **26**: 20.
- Fukuhara N. MERRF: a clinicopathological study. Relationships between myoclonus epilepsies and mitochondrial myopathies. *Rev Neurol (Paris)* 1991; **147**: 476.
- Berkovic SF, Carpenter S, Karpai G *et al*. Cytochrome c oxidase deficiency: a remarkable spectrum of clinical and neuropathological findings in a single family. *Neurology* 1987; **37**: 223.
- Berkovic SF, Andermann F, Karpai G *et al*. Mitochondrial encephalomyopathies: a solution to the enigma of the Ramsay-Hunt syndrome. *Neurology* 1987; **37**(Suppl. 1): 125.

23. Ogasahara S, Engel AG, Frens D, Mack D. Muscle coenzyme Q deficiency in familial mitochondrial encephalomyopathy. *Proc Natl Acad Sci USA* 1989; **86**: 2379.
24. DiMauro S, Hirano M, Bonilla E, De Vivo DC. The mitochondrial disorders. In: Berg BO (ed.). *Principles of Child Neurology*. New York: McGraw Hill, 1996: 1201.
25. Shoffner JM, Wallace DC. Oxidative phosphorylation diseases. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic and Molecular Bases of Inherited Disease*, 7th edn. New York: McGraw Hill, 1995: 1535.
26. Byrne E, Denner X, Trounce I, Burdon J. Mitochondrial myoneuropathy with respiratory failure and myoclonic epilepsy. A case report with biochemical studies. *J Neurol Sci* 1985; **71**: 273.
27. Mancuso M, Filosto M, Mootha VK *et al*. A novel mitochondrial tRNA-phe mutation causes MERRF syndrome. *Neurology* 2004; **62**: 2119.
28. Blakely EL, Trip SA, Swalwell H *et al*. A new mitochondrial transfer RNA(pro) gene mutation associated with myoclonic epilepsy with ragged-red fibers and other neurological features. *Arch Neurol* 2009; **66**: 399.
29. Takeda S, Ohama E, Ikuta F. Involvement of extraocular muscle in mitochondrial encephalomyopathy. *Acta Neuropathol* 1990; **80**: 118.
30. Berkovic SF, Andermann E, Carpenter S *et al*. Mitochondrial encephalomyopathies: evidence for maternal transmission. *Am J Hum Genet* 1987; **41**: A47.
31. Byrne E, Trounce I, Marzuki S *et al*. Functional respiratory chain studies in mitochondrial cytopathies. Support for mitochondrial DNA heteroplasmy in myoclonus epilepsy and ragged red fibers (MERRF) syndrome. *Acta Neuropathol (Berl)* 1991; **81**: 318.
32. Melone MAB, Tessa A, Petrini S *et al*. Revelation of a new mitochondrial DNA mutation (G12147A) in a MELAS/MERFF phenotype. *Arch Neurol* 2004; **61**: 269.
33. Zeviani ML, Muntoni F, Savarese N *et al*. A MERRF/MELAS overlap syndrome associated with a new point mutation of mitochondrial DNA tRNA-Lys gene. *Eur J Hum Genet* 1993; **1**: 80.
34. Berkovic SF, Shoubridge EA, Andermann F *et al*. Clinical spectrum of mitochondrial DNA mutation at base pair 8344. *Lancet* 1991; **338**: 457.
35. Eleff SM, Barker PB, Blackband SJ *et al*. Phosphorus magnetic resonance spectroscopy of patients with mitochondrial cytopathies demonstrates decreased levels of brain phosphocreatine. *Ann Neurol* 1990; **27**: 626.
36. Sasaki H, Kuzuhara S, Kanazawa I *et al*. Myoclonus cerebellar disorder neuropathy mitochondrial myopathy and ACTH deficiency. *Neurology* 1983; **33**: 1288.
37. Nakano T, Sakai H, Amano N *et al*. An autopsy case of degenerative type myoclonus epilepsy associated with Friedreich's ataxia and mitochondrial myopathy. *Brain Nerve* 1982; **34**: 321.
38. Fukuhara N. Myoclonus epilepsy and mitochondrial myopathy. In: Scarlato G, Cerri C (eds). *Mitochondrial Pathology in Muscle Diseases*. Padua: Pikin Medical Books, 1983: 88.
39. Sengers RCA, Stadhouders AM, Trijbels JMF. Mitochondrial myopathies: clinical, morphological and biochemical aspects. *Eur J Pediatr* 1984; **141**: 192.
40. DiMauro S, Bonilla E, Seiviani M *et al*. Mitochondrial myopathies. *Ann Neurol* 1985; **17**: 521.
41. Tanno Y, Tameda M, Nonaka I *et al*. Quantitation of mitochondrial DNA carrying tRNA^{Lys} mutation in MERRF patients. *Biochem Biophys Res Commun* 1991; **179**: 880.
42. Zeviani M, Amati P, Bresolin N *et al*. Rapid detection of the A to G(8344) mutation of mtDNA in Italian families with myoclonus epilepsy and ragged-red fibers (MERRF). *Am J Hum Genet* 1991; **48**: 203.
43. Seibel P, Degoul F, Bonne G *et al*. Genetic biochemical and pathophysiological characterization of a familial mitochondrial encephalomyopathy (MERRF). *J Neurol Sci* 1991; **105**: 217.
44. Noer AS, Sudoyo H, Lertrit P *et al*. A tRNA(Lys) mutation in the mtDNA is the causal genetic lesion underlying myoclonic epilepsy and ragged-red fiber (MERRF) syndrome. *Am J Hum Genet* 1991; **49**: 715.
45. Seibel P, Degoul F, Romero N *et al*. Identification of point mutations by mispairing PCR as exemplified in MERRF disease. *Biochem Biophys Res Commun* 1990; **173**: 561.
46. Chomyn A. The myoclonic epilepsy and ragged-red fiber mutation provides new insights into human mitochondrial function and genetics. *Am J Hum Genet* 1998; **62**: 745.
47. Silvestri G, Moraes CT, Shanske S *et al*. A new mutation in the tRNA-Lys gene associated with myoclonic epilepsy and ragged-red fibers (MERRF). *Am J Hum Genet* 1992; **51**: 1213.
48. Moraes CT, Ciacci F, Bonilla E *et al*. Two novel pathogenic mitochondrial DNA mutations affecting organelle number and protein synthesis. *J Clin Invest* 1993; **92**: 2906.
49. Nakamura M, Nakano S, Gato Y *et al*. A novel point mutation in the mitochondrial tRNA (ser(UCN)) gene detected in a family with MERRF/MELAS overlap syndrome. *Biochem Biophys Res Commun* 1995; **214**: 86.
50. Wong LJ, Yim D, Bai RK *et al*. A novel mutation in the mitochondrial tRNA (Ser(AGY)) gene associated with mitochondrial myopathy, encephalopathy, and complex 1 deficiency. *J Med Genet* 2006; **43**: e46.
51. Chomyn A, Meola G, Bresolin N *et al*. *In vitro* genetic transfer of protein synthesis and respiration defects to mitochondrial DNA-less cells with myopathy-patient mitochondria. *Mol Cell Biol* 1991; **11**: 2236.
52. Boulet L, Karpatis G, Shoubridge E. Distribution and threshold expression of the tRNA-Lys mutation in skeletal muscle of patients with myoclonic epilepsy and ragged-red fibers (MERRF). *Am J Hum Genet* 1992; **51**: 1187.
53. Riggs JE, Schochet SSJ, Fakadej AV *et al*. Mitochondrial encephalomyopathy with decreased succinate-cytochrome c reductase activity. *Neurology* 1984; **34**: 48.
54. Hutchinson CAI, Newbold JA, Potter SS, Edgell MH. Maternal inheritance of mammalian mitochondrial DNA. *Nature* 1974; **251**: 536.
55. Piko L, Matsumoto L. Number of mitochondria and some properties of mitochondrial DNA in the mouse egg. *Dev Biol* 1976; **49**: 1.

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56. Ruiters MHJ, van Spronsen EA, Skjeldal OH *et al.* Confocal scanning laser microscopy of mitochondria: a possible tool in the diagnosis of mitochondrial disorders. *J Inherit Metab Dis* 1991; **14**: 45.
 57. Wallace DC, Ye JH, Necklemann SN *et al.* Sequence analysis of cDNAs for the human and bovine ATP synthase beta subunit: mitochondrial DNA genes sustain seventeen times more mutations. *Curr Genet* 1987; **12**: 81.
 58. Larsson N-G, Tulinius MH, Holme E *et al.* Segregation and manifestations of the mtDNA tRNA-Lys A to G (8344) mutation of myoclonus epilepsy and ragged red fibers (MERRF) syndrome. *Am J Hum Genet* 1992; **51**: 1201.
 59. Oldfors A, Holme E, Tulinius M, Larsson N-G. Tissue distribution and disease manifestations of the tRNA^{Lys} A→G(8344) mitochondrial DNA mutation in a case of myoclonus epilepsy and ragged red fibers. *Acta Neuropathol* 1995; **90**: 328.

Neurodegeneration, ataxia, and retinitis pigmentosa (NARP)

Introduction	388	Treatment	391
Clinical abnormalities	388	References	392
Genetics and pathogenesis	391		

MAJOR PHENOTYPIC EXPRESSION

Neurodegeneration, ataxia, pigmentary retinopathy, Leigh syndrome, neurogenic muscle weakness, peripheral neuropathy, and point mutation in the mitochondrial gene for subunit 6 of mitochondrial adenosine triphosphatase (ATPase) (MTATP6), usually a T to G8993, or a T to C8993 transversion.

INTRODUCTION

The 8993 mutation was first described by Holt and colleagues [1] in a family with a maternally inherited neurodegenerative disease in three generations. The major phenotype was of neurogenic muscle weakness, ataxia, and retinitis pigmentosa, and this led to the acronym NARP. The 8993 T-to-G mutation is now referred to as the NARP mutation. In 1992, Tatuch and colleagues [2] reported the occurrence of this mutation in an infant who died at seven months and at autopsy had the typical neuropathology of Leigh syndrome. It is now clear that 8933 mutation is a common cause of Leigh syndrome [3]. A second mutation at position 8993 changing T to C in the ATPase 6 gene was identified in a family with Leigh syndrome [4]. It was noted early that the percentage of mutant mitochondrial DNA varied considerably in heteroplasmic affected individuals, and this leads to considerable variability in phenotypic expression [5]. From the initial description, there was correlation between the amount of mutant DNA and the severity of clinical manifestations [1].

CLINICAL ABNORMALITIES

The index family was recognized as having a mitochondrial disease not previously described in which there was a variable combination of retinitis pigmentosa, neurogenic

proximal muscle weakness, ataxia, sensory neuropathy, developmental delay, seizures, and dementia. There were four patients in three generations. The initial patient was a 47-year-old woman who developed night blindness at 12 years of age and was found to have retinitis pigmentosa; she was nearly blind by 30 years. At 24 years, she had a grand mal seizure and was treated with phenytoin. Unsteadiness in walking was progressive in her thirties. On examination, she had marked ataxia. Ankle jerks were absent and proprioceptive and pain sensations were diminished in the distal lower extremities. Nerve conduction velocity was reduced. Her asymptomatic sister had clumps of retinal pigment and proximal muscle weakness. The daughter of this sister had reduced vision at 25 years and retinitis pigmentosa on examination along with mild proximal muscle weakness and ataxia, and extensor plantar responses. Her second daughter developed normally until she had a febrile illness at 28 months, in which she was unwell for a month, and she then stopped walking for five months. At three years, she spoke only single words and had pigmentary retinopathy. She was ataxic and had increased tone in the limbs, exaggerated deep tendon reflexes and extensor plantar responses. The electroencephalograph (EEG) was abnormal.

Night blindness is often the first symptom of these patients [6]. This is followed by loss of peripheral vision and, in some, loss of central vision. The index patient at 47 years could just perceive light. Examination of the retina

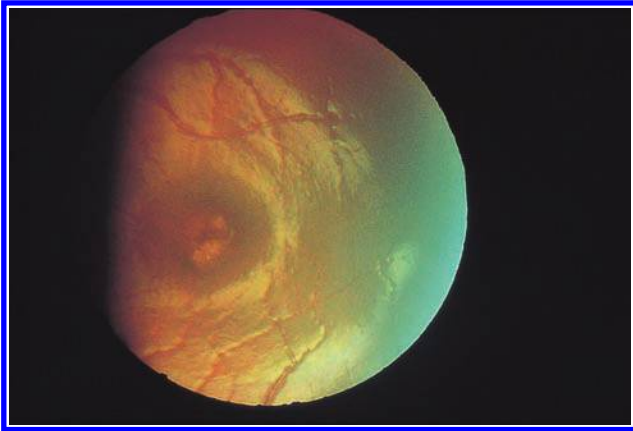


Figure 53.1 AA: A patient with adenosine triphosphatase (ATP) synthase deficiency had pigmentary degeneration of the retina.

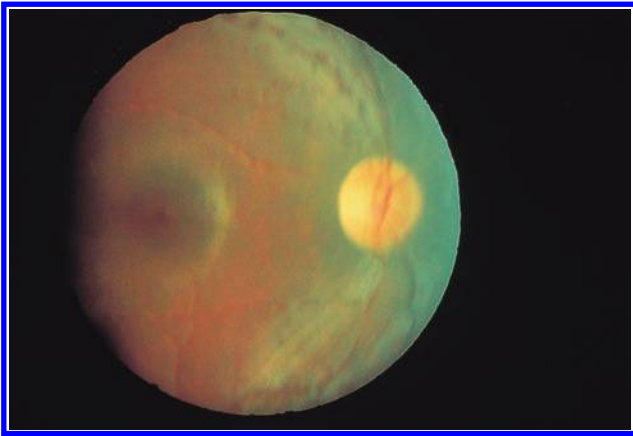


Figure 53.2 AA: The other fundus was also involved. His mutation was in peptide 6.

reveals evidence of retinitis pigmentosa (Figures 53.1 and 53.2). The appearance of the clumps of pigment in the retina typically resembles spicules of bone [1, 7]. Some retinas may have a salt-and-pepper appearance [8]. The electroretinogram may be abnormal, as may visual fields. Others have optic atrophy [9]. There may be nystagmus on horizontal or vertical gaze and esotropia [8]. Progression of retinal disease has been described from the appearance of salt and pepper in the retina in the absence of symptoms to constriction of visual fields along with the appearance of bone spicules in the retina, optic nerve pallor, and arteriolar attenuation.

Ataxia may be a prominent feature of the disease (Figures 53.3, 53.4, and 53.5). It may lead to injuries and localized areas of traumatic hyperplasia. Cerebellar atrophy has been observed on neuroimaging [9]. Other patients have had dystonia.

Some patients have been impressively hypotonic [9]. Others have had localized proximal muscle weakness, but as recognized in the initial series the weakness is neurogenic. Muscle biopsy does not show ragged red fibers



Figure 53.3 BF: A girl with the neurodegeneration, ataxia, and retinitis pigmentosa (NARP) mutation, a deletion at 8993, in mitochondrial DNA. She had been well until approximately a year before when she and her twin brother developed an acute life-threatening episode of which he died.



Figure 53.4 BF: She was quite ataxic and fell so frequently she had a raised bony area in her mid-forehead.

or abnormal mitochondria [1–3, 9–12]. There may be evidence of denervation, lipid droplets, or variation in fiber size diameter. The electromyogram (EMG) is normal.

Peripheral neuropathy may be evident on clinical examination [1]. Nerve conduction velocity is reduced in a pattern of axonal sensory neuropathy.

Seizures may be generalized and associated with spike and wave bursts on EEG [1, 9]. Two patients have had infantile spasms and an EEG pattern of hypsarrhythmia [9]. Others have had myoclonic seizures. Less severely affected patients have had migraine, some with no other

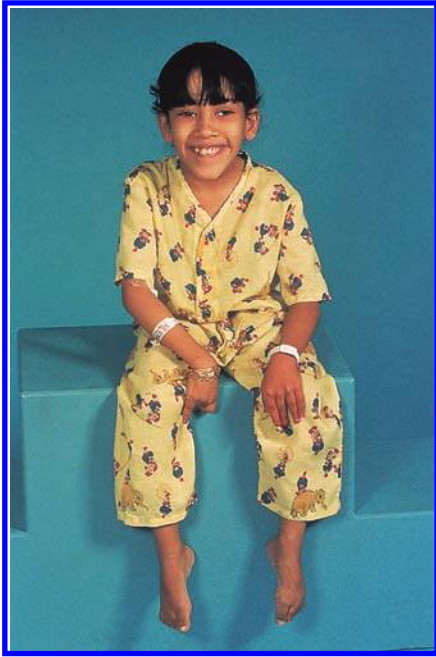


Figure 53.5 TS: A patient with the neurodegeneration, ataxia, and retinitis pigmentosa (NARP) syndrome. She had ataxia and retinitis pigmentosa.

manifestation of illness. Others have had depression or bulimia. Some have been developmentally delayed, some of them severely. Hearing loss has been reported [13].

There have been a number of deaths in infancy (Figure 53.3) [2, 9]. The acute life-threatening episode may be associated with lactic acidemia [2]. The advent of dichloroacetate control of lactic acidemia has permitted us to observe episodes of acute acidemias in patients with the NARP mutation in the absence of lactic acidemia. These episodes of acute acidosis requiring admission to hospital, and parenteral fluid and electrolyte therapy have been characterized by ketoacidosis. An infant who died had lactate levels of 3–5 mmol/L [2]. Others had levels up to 5.2 mmol/L [9]. In one 50-year-old man, with mildly impaired mental development the cerebrospinal fluid lactate was normal [9]. Cerebrospinal fluid concentrations of protein were normal [9]. Some patients have had recurrent vomiting.

An expanded spectrum of NARP syndrome [14] was recognized when the 8993G mutation was found in a patient whose diagnosis on magnetic resonance imaging (MRI) was acute demyelinating encephalomyelitis (ADEM). The patient had neurogenic muscle weakness, and ataxia, typical for NARP, but other affected family members had fewer clinical manifestations. Nevertheless, his mother died of fulminant hepatic failure following valproate administration. In four families studied, none had retinitis pigmentosa. One was referred for ‘cerebral palsy’, attention deficit disorder, and learning disability. Later, he had episodes of ataxia, headache, and peripheral neuropathy following febrile illnesses.

Leigh syndrome (Chapter 46) was first associated with

the classic NARP mutation in the autopsy of an infant who died at seven months of age with lactic acidemia, seizures, and apnea [9]. She had been hypotonic and had head lag from early infancy. Neuropathologic examination revealed bilateral cystic lesions of the basal ganglia, thalamus, substantia nigra, and tegmental brainstem. There was proliferation of astrocytes and blood vessels in these areas. A maternal aunt and uncle had died of Leigh syndrome. Another maternal uncle was normal until 12 years of age, when he developed a bout of weakness and ataxia from which he recovered. There were further episodes and he developed retinitis pigmentosa. At 33 years, he was ataxic, legally blind, mentally handicapped, and in an institution.

Of seven patients with typical Leigh spongiform changes on neuropathology or the characteristic appearance on MRI, the classic NARP mutation was found in all [15]. In each, there was heteroplasmy, but the mutation was in high proportion in blood and muscle. It was found in four asymptomatic mothers and two asymptomatic siblings. This series was expanded to 12 patients in ten families, all with the same mutation [3]. Consistent with the observations of Tatuch *et al.* [2], the Leigh phenotype was associated with a high percentage of abnormal mitochondrial DNA. The heterogeneity of Leigh syndrome is pointed up by the fact that the 12 patients of Santorelli *et al.* [3] were found in the study of 50 patients with typical Leigh syndrome. These authors compared 18 patients reported with the NARP mutation and Leigh syndrome with 34 and 64 in whom the underlying disease was cytochrome oxidase deficiency or pyruvate dehydrogenase complex deficiency (Chapter 49), respectively. Smaller numbers had biotinidase or complex I deficiency. Among the features of the clinical picture, only retinitis pigmentosa and positive family history seemed to distinguish the patients with NARP. An earlier-onset, more rapid course or propensity for seizures was more common among the NARP patients. Patients with the classic NARP mutation and Leigh syndrome were also reported by Ciafaloni and colleagues [11], Shoffner and colleagues [8], and Mäkelä-Bengs and colleagues [9]. Thus, it is clear that the 8993 mutation in mitochondrial DNA is a common cause of Leigh syndrome.

The syndrome in these patients is characterized by developmental delay, some after a period of normal development, and hypotonia followed by psychomotor regression; some have had ataxia or dystonic posturing [8]. Spastic quadriparesis has been reported [3]. Brainstem dysfunction leads to ophthalmoplegia, apnea, ventilator dependence, or death [3]. Neuroimaging reveals symmetric areas of decreased density on computed tomography (CT) or MRI in the basal ganglia, periventricular and periaqueductal areas (Figure 53.6). Blood concentrations of lactate were increased as high as 7 mmol/L with a mean concentration of 4.6 mmol/L [3]. A cerebrospinal fluid concentration of 7.12 mmol/L was reported [11]. Neuropathological examination revealed reduction in the size of the caudate, globus pallidus, putamen, and cerebellum. Microscopic examination showed gliosis and

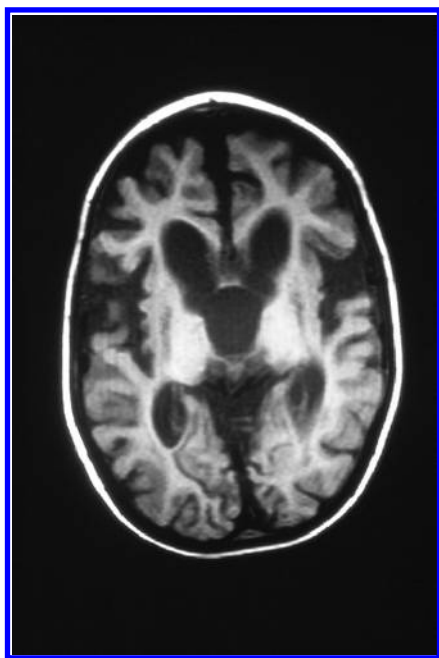


Figure 53.6 AA: Computed tomographic scan of the brain. There was considerable atrophy.

demyelination of white matter and spongiform changes with relative preservation of neurons in the basal ganglia, thalamus, hypothalamus, and medulla [8]. Some patients have had hypertrophic cardiomyopathy [3].

GENETICS AND PATHOGENESIS

The NARP mutation is clearly inherited in a maternal pattern [11, 12, 14–17]. Recurrence of disease in two siblings of a mother with two different mates has been reported [11]. All of the families studied have revealed degrees of heteroplasmy. In general, the correlation between the severity of phenotype and the proliferation of mutant mitochondrial DNAs has been very good [1–3, 9, 11]. Patients with infantile encephalopathy and Leigh syndrome have tended to have over 95 percent of mutant mitochondrial DNA. Later-onset patients have had 80–90 percent, while asymptomatic patients may have had as little as 3–6 percent. Correlation has been better with the proportion of mutant DNA in the blood than in the fibroblast. For instance, an asymptomatic mother had 39 percent in blood and 71 percent in fibroblasts [2].

Prenatal diagnosis has been made in two affected pregnancies at risk by examination of chorionic villus samples [18], but, because of the random distribution of mutant DNA, neither prenatal diagnosis nor carrier detection is reliable. Calculations from 56 pedigrees relating severity of symptoms and mutant load should be useful in genetic counseling [5].

There has been some evidence for nonrandom segregation of mutant NARP mitochondria in oocytes,

such as a mother with 10 percent mutant DNAs having three offspring with 90 percent or more mutant DNAs. Examination of oocytes has revealed a predominance of mutant DNA. A bottleneck for mitochondrial DNA in embryogenesis might lead to a reduction or enhancement of amounts of mutant mitochondrial DNA [9].

The common mutation was found originally in nucleotide 8993 of the mitochondrial genome when digestion of leukocyte mitochondrial DNA with the restriction endonuclease *AvaI* revealed an unusual pattern of fragments. In involved members, a variable portion of the normal 14.4-kb fragment was cleaved into two, one 10.4 kb and one 4.0 kb. Further digestion with *PvuII* cleaved the 10-kb fragment into two of 6 and 4 kb, respectively, which localized the gain of the *AvaI* site to the ATPase 6 reading frame. Both normal and mutant populations were found in muscle, as well as blood. Polymerase chain reaction (PCR) amplification with primers 8648–8665 and 9180–9199 revealed a single fragment, which was cleaved by *AvaI* in patients, but not in controls. Sequencing identified the T-to-G change at 8993. This change leads to a change from the highly conserved hydrophobic leucine to the hydrophilic arginine at position 156 of subunit 6 of the mitochondrial H^+ -ATPase. This would be expected to interfere with the hydrogen ion channel formed by subunits 6 and 9 and lead to failure of adenosine triphosphatase (ATP) synthesis [2]. It did not affect hydrolysis of ATP. A *de novo* insertion in the *MT-ATP* gene led to a truncated subunit and a decrease in the amount of ATP synthase [13]. The patient, a 40-year-old with blindness and optic atrophy was heteroplasmic, 85 percent in muscle and 26 percent in blood.

The activity of the enzymes of the respiratory chain tend to be normal in the frozen muscle of patients [3]. However, Shoffner and colleagues [8] found deficiencies in the activities in muscle of complex I and III. Testing of oxidative phosphorylation revealed a reduction in the rate of generation of ATP [9]. The T-to-C mutation at 8993 replaces the leucine with proline. This would change the helical structure of the protein and would be expected to interfere with proton conduction [19]. Nevertheless, ATP production was not impaired [20]. In general, the 8993C disease tends to be less severe than the 8993G [5].

Cultured fibroblasts with the 8993G genotype were protected from death in the presence of gramicidin or oligomycin in a glucose-free medium by incubation with 2-oxoglutarate/aspartate, which increased ATP content [21]. Patients have not yet been treated.

TREATMENT

Supportive treatment includes the management of seizure disorder and the prompt treatment of infection. Experience with dichloroacetic acid indicates success in reducing levels of lactic acid. Effects on the neurologic features of the disease were unimpressive.

REFERENCES

- Holt IJ, Harding AE, Petty RK, Morgan-Hughes JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. *Am J Hum Genet* 1990; **46**: 428.
- Tatuch Y, Christodoulou J, Feigenbaum A *et al*. Heteroplasmic mtDNA mutation (T→G) at 8993 can cause Leigh disease when the percentage of abnormal mtDNA is high. *Am J Hum Genet* 1992; **50**: 852.
- Santorelli FM, Shanske S, Macaya A *et al*. The mutation at nt 8993 of mitochondrial DNA is a common cause of Leigh's syndrome. *Ann Neurol* 1993; **34**: 827.
- de Vries DD, van Engelen BGM, Gabreëls FJM *et al*. A second missense mutation in the mitochondrial ATPase 6 gene in Leigh's syndrome. *Ann Neurol* 1993; **34**: 410.
- White SL, Collins VR, Wolfe R *et al*. Genetic counseling and prenatal diagnosis for the mitochondrial DNA mutations at nucleotide 8993. *Am J Hum Genet* 1999; **65**: 474.
- Shoffner JM, Wallace DC. Oxidative phosphorylation diseases. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic and Molecular Bases of Inherited Disease*. New York: McGraw Hill, 1995: 1535.
- Ortiz RG, Newman NJ, Shoffner JM *et al*. Variable retinal and neurologic manifestations in patients harbouring the mitochondrial DNA 8993 mutation. *Arch Ophthalmol* 1993; **111**: 1525.
- Shoffner JM, Fernhoff PM, Krawiecki NS *et al*. Subacute necrotizing encephalopathy: oxidative phosphorylation defects and the ATPase 6 point mutation. *Neurology* 1993; **42**: 2168.
- Mäkelä-Bengs P, Suomalainen A, Majander A *et al*. Correlation between the clinical symptoms and the proportion of mitochondrial DNA carrying the 8993 point mutation in the NARP syndrome. *Pediatr Res* 1995; **37**: 634.
- Kerrison JB, Biousse V, Newman NJ. Retinopathy of NARP syndrome. *Arch Ophthalmol* 2000; **118**: 298.
- Ciafaloni E, Santorelli FM, Shanske S *et al*. Maternally inherited Leigh syndrome. *J Pediatr* 1993; **122**: 419.
- Yoshinaga H, Ogino T, Ohtahara S *et al*. A T-to-G mutation at nucleotide pair 8993 in mitochondrial DNA in a patient with Leigh's syndrome. *J Child Neurol* 1993; **8**: 129.
- Lopez-Gallardo E, Solano A, Herrero-Martin MD *et al*. NARP syndrome in a patient harbouring an insertion in the MT-ATP6 gene that results in a truncated protein. *J Med Genet* 2009; **45**: 64.
- McGowan KA, Naviaux RK, Barshop BA *et al*. The expanding clinical spectrum of the NARP syndrome. *J Invest Med* 1998; **46**: 86A.
- Santorelli FM, Shanske S, Jain KD *et al*. A new mtDNA mutation in the ATPase 6 gene in a child with Leigh syndrome. *Neurology* 1993; **43**: A171.
- Santorelli FM, Shanske S, Sciacco M *et al*. A new etiology for Leigh syndrome: mitochondrial DNA mutation in the ATPase 6 gene. *Ann Neurol* 1992; **32**: 467 (Abstr. 141).
- Puddo P, Barboni P, Mantovani V *et al*. Retinitis pigmentosa ataxia and mental retardation associated with mitochondrial DNA mutation in an Italian family. *Br J Ophthalmol* 1993; **77**: 84.
- Harding AE, Holt IJ, Sweeney MG *et al*. Prenatal diagnosis of mitochondrial DNA 8993 T→G disease. *Am J Hum Genet* 1992; **50**: 629.
- Cox GB, Fimmel AL, Gibson F, Hatch L. The mechanism of ATP synthase: a reassessment of the function of the a and b subunits. *Biochim Biophys Acta* 1986; **849**: 62.
- Anderson S, Bankier AT, Barrell BG *et al*. Sequence and organization of the human mitochondrial genome. *Nature* 1981; **290**: 457.
- Sgarbi G, Casalena GA, Baracca A *et al*. Human NARP mitochondrial mutation metabolism corrected with alpha-ketoglutarate/aspartate: a potential new therapy. *Arch Neurol* 2009; **66**: 951.

Kearns-Sayre syndrome

Introduction	393	Treatment	396
Clinical abnormalities	393	References	396
Genetics and pathogenesis	395		

MAJOR PHENOTYPIC EXPRESSION

Onset prior to 20 years of age of progressive external ophthalmoplegia, ptosis, pigmentary retinopathy, block in cardiac conduction, ataxia, elevated protein in CSF, ragged red muscle fibers, and deletion in mitochondrial DNA.

INTRODUCTION

In 1995, Kearns and Sayre [1] reported a syndrome of retinitis pigmentosa, external ophthalmoplegia, and complete heart block. It has for some time been recognized as an encephalomyopathy with variable neurologic manifestations, including cerebellar ataxia, muscle weakness, sensorineural deafness, and mental deterioration [2]. There may be elevation of the protein in cerebrospinal fluid (CSF) to values over 100 mg/dL. Muscle biopsy reveals ragged red fibers [3]. Lestienne and Ponsot [4], Holt and colleagues [5], and Zeviani *et al.* [6], in 1988, reported deletions in the DNA of mitochondria in biopsied muscle. The common deletion approximates 5 kb. The disease is virtual always the result of spontaneous new mutation.

CLINICAL ABNORMALITIES

Patients with Kearns-Sayre syndrome usually appear normal in early childhood, developing features of the disease in later childhood or adolescence. It is probably an artificial distinction that patients developing signs of disease after the age of 20 years are referred to as having chronic progressive external ophthalmoplegia (PEO), because they may have the same deletions as those presenting earlier and may develop any of the multisystem features of classic Kearns-Sayre syndrome [2].

The earliest manifestation is often a limitation of external ocular movement or ptosis (Figures 54.1 and 54.2). These manifestations are chronically progressive and the classic



Figure 54.1 GF: A ten-year-old girl with Kearns-Sayre syndrome. She was very short and had pronounced ptosis bilaterally.

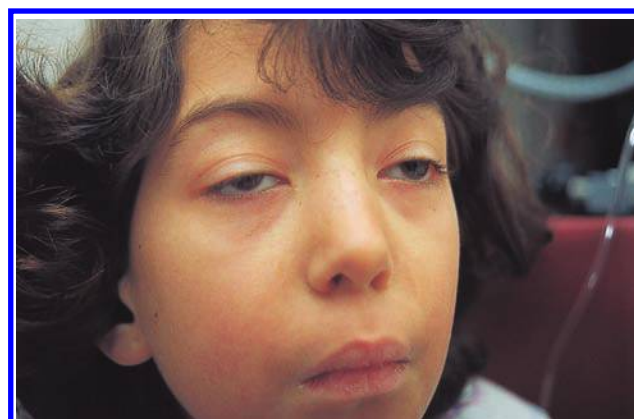


Figure 54.2 GF: When asked to follow a light upwards, the patient demonstrated a paresis of upward gaze.

appearance is that of bilateral ptosis and ophthalmoplegia. There may be progression to complete ophthalmoplegia. Electromyography (EMG) of the orbicularis oculi muscle may show myogenic changes.

Pigmentary degeneration of the retina may take the pattern of salt and pepper retinopathy in which there are regions of hyper- and hypopigmentation or a bone spicule appearance of retinitis pigmentosa [7, 8]. The Kearns-Sayre triad is ptosis, ophthalmoplegia, and pigmentary retinopathy. There may be optic atrophy. Some patients have had an eventual loss of the pigment epithelium. Others have had a choroideremia pattern in which there is complete choroid atrophy [9]. Visual impairment may be the presenting complaint. Other patients with pigmentary changes on fundoscopy may have normal visual acuity. Electroretinopathy may reveal delayed A waves signifying tapetoretinal degeneration even in patients without symptoms or abnormality visible in the fundus.

Skeletal myopathy may be evident in muscle weakness or exercise intolerance. Deep tendon reflexes may be diminished. Some patients have developed scoliosis. Cerebellar abnormality may be evident in ataxia, a broad-based gait, or dysmetria. There may be an intention tremor. Sensorineural deafness is another common neurologic manifestation of the disease. Dementia may ultimately occur. Muscle biopsy classically reveals ragged red fibers [10] when the specimen is stained with Gomori trichrome. Structural abnormality may be identified by electron microscopy. There may be aggregates of mitochondria [11].

Cerebrospinal fluid concentration of protein is usually referred to as >100 mg/dL, but many patients, even among those with elevated concentrations of protein, have lower levels [10]. Computed tomography (CT) scan or magnetic resonance imaging (MRI) may reveal atrophy of the cerebellum or brain stem [12], and there may be calcifications in the basal ganglia [12, 13]. Some patients have had lesions in the thalamus and brain stem as seen in Leigh syndrome. Others have had diffuse white matter hypodensities [6]. The histopathology of the brain is that of spongiform degeneration [10].

Cardiac conduction (Figure 54.3) is classically abnormal and typically takes the form of a complete atrioventricular block or a right bundle branch block. The PQ interval may be prolonged on electrocardiogram (ECG), or there may be a prolonged QT [13]. Evidence of cardiomyopathy has been obtained by biopsy [11]. Clinical evidence of cardiomyopathy can range from tachycardia to frank failure. One of our pediatric patients presented with a seizure resulting from complete heart block [14]. She also had retinopathy and ophthalmoplegia.

Other nonneurologic manifestations include shortness of stature. A variety of endocrine abnormalities have been encountered [2, 15], the most common of which are diabetes mellitus and hypogonadism, including amenorrhea and delayed puberty. Hypoparathyroidism, thyroid abnormalities, and hyperaldosteronism are less frequent [7, 16, 17]. Nonautoimmune Addison disease has been reported [17] in

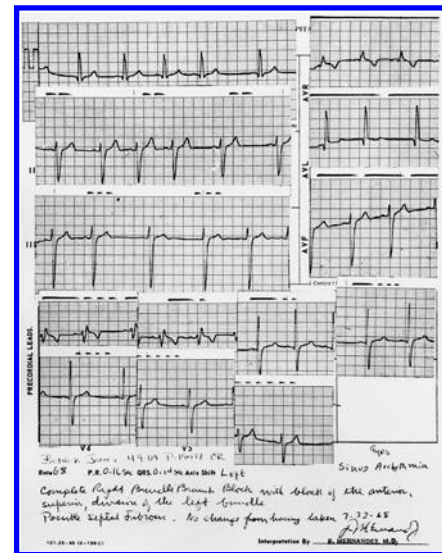


Figure 54.3 Electrocardiogram of a patient with Kearns-Sayre syndrome, illustrating a complete heart block.

a patient with a 4.9-kb deletion. Hypomagnesemia has been observed [13]. Hypoparathyroidism has been a component of a multiple endocrine abnormality syndrome [18].

An 18-year-old girl was reported [19] in whom hypoparathyroidism and renal tubular dysfunction were associated with inappropriate hyperexcretion of magnesium and potassium. Another hypoparathyroid patient presented at five years of age with recurrent episodes of carpopedal spasms resulting from persistent hypomagnesemia and hypocalcemia with increased urinary fractional excretion of magnesium and calcium [20].

Another syndrome has been reported in four unrelated children with Kearns-Sayre syndrome who presented first with hypoparathyroidism and deafness [13]. Hypocalcemic tetany, a consequence of deficiency of parathyroid hormone, was well controlled by treatment with low doses of 1,25-dihydroxychole calciferol. Two of three patients had hypomagnesemia.

Renal tubular acidosis is another interesting manifestation [20, 21] which is also seen in other disorders of mitochondrial electron transport function. There may be glycosuria and a generalized amino aciduria [20]. Some presentations have resembled Bartter syndrome or Lowe syndrome [22].

Lactic acidemia is not a predominant feature in many patients, but some have had lactic acidosis; some have mild elevations of levels of lactic acid in the blood. In others, the CSF concentration of lactic acid is elevated in the absence of lactic acidemia.

An unusual presentation is with the Pearson-marrow syndrome (Chapter 55). Patients with the Pearson syndrome have the same area of deletion as Kearns-Sayre syndrome, and patients who have survived the early morbidity of the Pearson syndrome and whose marrow dysfunction resolved have been reported to go on to develop Kearns-Sayre syndrome [23].

GENETICS AND PATHOGENESIS

Kearns-Sayre syndrome is virtually always sporadic [10], suggesting that the deletion in the mitochondrial genome occurred in the formation of the affected individual. Many mothers of affected individuals have been studied without finding deletions. A few pedigrees of patients with Kearns-Sayre or chronic progressive ophthalmoplegia syndromes have displayed patterns of inheritance consistent with an autosomal dominant gene [2, 24–27]. Analysis of mitochondrial DNA has revealed multiple deletions in muscle. These deletions could not be found in rapidly dividing tissues, such as leukocytes or cultured fibroblasts. It has been postulated that an abnormal mutant gene has led to the mutations in the mitochondrial genome. A similar pattern of multiple deletions in mitochondrial DNA was seen in a study in which there were two affected brothers and first cousin parents, a pattern suggesting autosomal recessive inheritance [28, 29].

The vast majority of patients with Kearns-Sayre syndrome have deletions spanning approximately 5 kb and referred to as the common deletion (Figures 54.4 and 54.5). Many different deletions have been observed in the O_H to O_L arc (Figure 55.1). In about half of patients, this is a 4.9-kb deletion extending from the NAD dehydrogenase (ND5) to the ATPase subunit gene [30]. This is an area in which there are 13-bp direct repeats on either side, np 13,447 to 13,459 and np 8470 to 8482 [31–33]. It appears likely that this produces a situation in which hot spots promote deletion. Deletions in this area remove structural genes and some tRNA genes, which would interfere with mitochondrial protein synthesis. Overall deletions have ranged from 1.3 to 7.6 kb [16, 17]. The proportion of mutated genomes ranged in these series from 27 to 85 percent of total mitochondrial DNA. In some patients, the proportions in different tissues were very variable. In general, the proportion of abnormal DNA increases with age, paralleling the worsening of clinical manifestations. The deletions are all large enough to be readily distinguished from control by digestion with restriction endonucleases and electrophoresis on agarose gel (Figure 54.5). Southern blots display a 16.5-kb band in normal individuals and smaller bands in those with deletions.

A 4.9-kb deletion and heteroplasmy has been observed

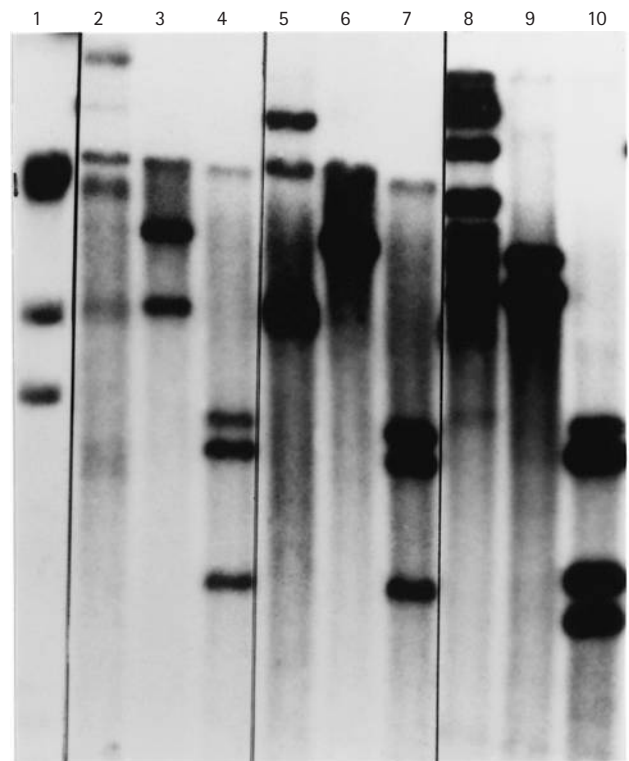


Figure 54.4 Electrophoretic patterns of restriction fragments of mitochondrial DNA from two patients with Kearns-Sayre syndrome (lanes 2–4 and 8–10 and a control individual, lanes 5–7). Lanes 2, 5, and 8 were uncut, lanes 3, 6, and 9 cut with *Bam*HI, and lanes 4, 7, and 9 were cut with both *Bam*HI and *Eco*RV. Lane 1 represented size standards. The patient shown in lanes 8–10 had the most commonly encountered deletion. (Reprinted from *Molecular Genetics and Metabolism* [14] with permission from Elsevier).

in wild mice [34]. It has been thought that deletions in a region between two areas of direct repeats could occur through slip-replication, in which, following a break at the first direct repeat the first repeat pairs with the second direct repeat (Figure 54.6).

In some patients, the abnormality in mitochondrial DNA is a duplication [35, 36]. In most instances, the clinical presentation is no different from that of patients with

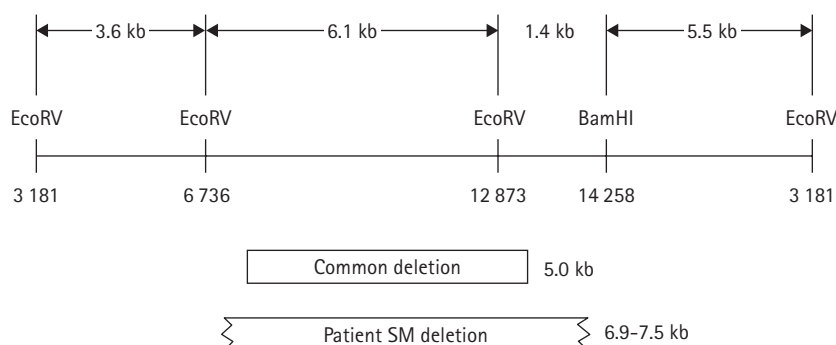


Figure 54.5 Linear representation of the common deletion and the deletion in a patient who presented first with 2-oxoadipic aciduria [14].

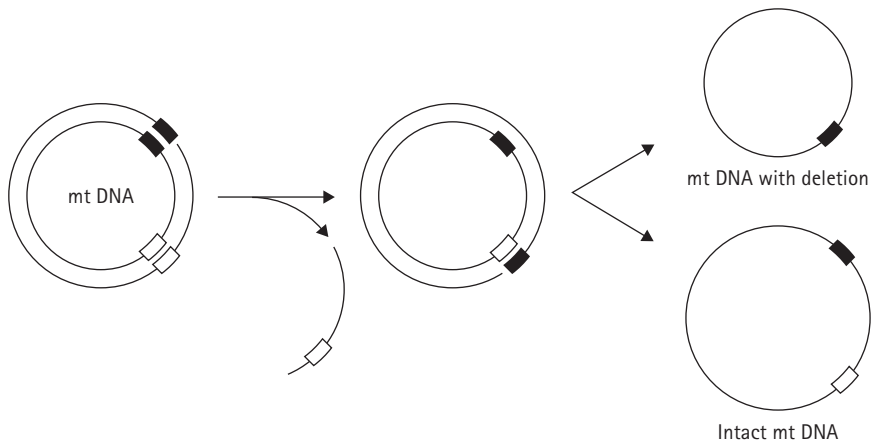


Figure 54.6 Slip replication model for the genesis of a deletion in mitochondrial DNA. Following the development of breaks, the smaller segment is removed and degraded.

deletions. Some have had renal tubular acidosis or other renal tubular abnormality [21, 22]. The children reported [13] with hypoparathyroidism and deafness in Kearns-Sayre syndrome had duplications, as well as deletions. The deletions all spared four genes of complex I, including ND3 and ND6, as well as both genes of complex V, ATPase 8 and 6. The sizes of the duplications were inversely proportional to the sizes of the deletions. Homologous recombination, as well as slip replication, could be the mechanism of these rearrangements [37, 38].

Although it is unusual, maternally inherited mitochondrial rearrangement can be seen in this disease [39].

Profound deficiency of cerebral folate concentration, despite normal serum concentrations was found in a patient with a large deletion [40].

TREATMENT

Coenzyme Q10 may be of benefit. Treatment with 60–120 mg daily was reported [41] to be associated with decrease in modestly elevated levels of lactic and pyruvic acids and improvement in the prolongation of the PQ interval on the ECG, as well as ocular movements. The QRS complex did not change. Concentrations of folic acid and of carnitine may be reduced in plasma or muscle, and treatment with these agents may be useful. A vitamin B complex supplement is often prescribed. The patient with cerebral folate deficiency was successfully treated with folic acid [40].

In the presence of complete A–V block, a cardiac pacemaker is usually required. Corrective eyeglasses may be helpful.

REFERENCES

1. Kearns T, Sayre GP. Retinitis pigmentosa, external ophthalmoplegia, and complete heart block. *Arch Ophthalmol* 1958; **60**: 280.
2. Berenberg RA, Pellock JM, DiMauro S *et al*. Lumping or splitting? 'Ophthalmoplegia-plus' or Kearns-Sayre syndrome? *Ann Neurol* 1977; **1**: 37.
3. DiMauro S, Bonilla E, Zeviani M *et al*. Mitochondrial myopathies. *Ann Neurol* 1985; **17**: 512.
4. Lestienne P, Ponsot G. Kearns-Sayre syndrome with muscle mitochondrial DNA deletion. *Lancet* 1988; **1**: 885 (letter).
5. Holt IJ, Harding AE, Morgan-Hughes JA. Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. *Nature* 1988; **331**: 717.
6. Zeviani M, Moraes CT, DiMauro S *et al*. Deletions of mitochondrial DNA in Kearns-Sayre syndrome. *Neurology* 1988; **38**: 1339.
7. Petty RK, Harding AE, Morgan HJA. The clinical features of mitochondrial myopathy. *Brain* 1986; **109**: 915.
8. Mullie MA, Harding AE, Petty RK *et al*. The retinal manifestations of mitochondrial myopathy. A study of 22 cases. *Arch Ophthalmol* 1985; **103**: 1825.
9. Herzberg NH, van Schooneveld MJ, Bleeker-Wagemakers EM *et al*. Kearns-Sayre syndrome with a phenocopy of choroideremia instead of pigmentary retinopathy. *Neurology* 1993; **43**: 218.
10. Rowland LP, Blake DM, Hirano M *et al*. Clinical syndromes associated with ragged red fibers. *Rev Neurol* 1991; **147**: 467.
11. Bastiaansen LAK, Joosten EMG, de Rooij JAM *et al*. Ophthalmoplegia-plus, a real nosological entity. *Acta Neurol Scand* 1978; **58**: 9.
12. Robertson WC Jr, Viseskul C, Lee YE, Lloyd RV. Basal ganglia calcification in Kearns-Sayre syndrome. *Arch Neurol* 1979; **36**: 711.
13. Wilichowski E, Gruters A, Kruse K *et al*. Hypoparathyroidism and deafness associated with pleioplasmic large scale rearrangements of the mitochondrial DNA: a clinical and molecular genetic study of four children with Kearns-Sayre syndrome. *Pediatr Res* 1997; **41**: 193.
14. Barshop BA, Nyhan WL, Naviaux RK *et al*. Kearns-Sayre syndrome presenting as 2-oxoadipic aciduria. *Mol Genet Metab* 2000; **69**: 64.
15. Quade A, Zierz S, Klingmüller D. Endocrine abnormalities in mitochondrial myopathy with external ophthalmoplegia. *Clin Invest* 1992; **70**: 396.

16. Moraes CT, DiMauro S, Zeviani M *et al.* Mitochondrial DNA deletions in progressive external ophthalmoplegia and Kearns-Sayre syndrome. *N Engl J Med* 1989; **320**: 1293.
17. Boles RG, Roe T, Senadheera D *et al.* Mitochondrial DNA deletion with Kearns Sayre syndrome in a child with Addison disease. *Eur J Pediatr* 1998; **157**: 643.
18. Harvey JN, Barnett D. Endocrine dysfunction in Kearns-Sayre syndrome. *Clin Endocrinol* 1992; **37**: 97.
19. Katsanos KH, Elisaf M, Bairaktari E *et al.* Severe hypomagnesemia and hypoparathyroidism in Kearns-Sayre syndrome. *Am J Nephrol* 2001; **21**: 150.
20. Lee YS, Yap HK, Barshop BB *et al.* Mitochondrial tubulopathy: the many faces of mitochondrial disorders. *Pediatr Nephrol* 2001; **16**: 710.
21. Eviatar L, Shanske S, Gauthier B *et al.* Kearns-Sayre syndrome presenting as renal tubular acidosis. *Neurology* 1990; **40**: 1761.
22. Moraes CT, Zeviani M, Schon EA *et al.* Mitochondrial DNA deletion in a girl with manifestations of Kearns-Sayre and Lowe syndromes: an example of phenotypic mimicry? *Am J Med Genet* 1991; **41**: 301.
23. Norby S, Lestienne P, Nelson I *et al.* Juvenile Kearns-Sayre syndrome initially misdiagnosed as a psychosomatic disorder. *J Med Genet* 1994; **31**: 45.
24. Zeviani M, Servidei S, Gellera C *et al.* An autosomal dominant disorder with multiple deletions of mitochondrial DNA starting at the D-loop region. *Nature* 1989; **339**: 309.
25. Barron SA, Heffner RRJ, Zwirecki R. A familial mitochondrial myopathy with central defect in neural transmission. *Arch Neurol* 1979; **36**: 553.
26. McAuley FD. Progressive external ophthalmoplegia. *Br J Ophthalmol* 1956; **40**: 686.
27. Zeviani M. Nucleus-driven mutations of human mitochondrial DNA. *J Inherit Metab Dis* 1992; **15**: 456.
28. Mizusawa H, Watanabe M, Kanazawa I *et al.* Familial mitochondrial myopathy associated with peripheral neuropathy: Partial deficiencies of complex I and complex IV. *J Neurol Sci* 1988; **86**: 171.
29. Yuzaki M, Ohkoshi N, Kanazawa I *et al.* Multiple deletions in mitochondrial DNA at direct repeats of non-D-loop regions in cases of familial mitochondrial myopathy. *Biochem Biophys Res Commun* 1989; **164**: 1352.
30. Wallace DC, Lott MT, Torroni A, Brown MD. Report of the committee on human mitochondrial DNA. *Cytogenet Cell Genet* 1992; **59**: 727.
31. Schon EA, Rizzuto R, Moraes CT *et al.* A direct repeat is a hotspot for large-scale deletion of human mitochondrial DNA. *Science* 1989; **244**: 346.
32. Shoffner JM, Lott MT, Voljavec AS *et al.* Spontaneous Kearns-Sayre/chronic external ophthalmoplegia plus syndrome associated with a mitochondrial DNA deletion: a slip-replication model and metabolic therapy. *Proc Natl Acad Sci USA* 1989; **86**: 7952.
33. Johns DR, Rutledge SL, Stine OC, Hurko O. Directly repeated sequences associated with pathogenic mitochondrial DNA deletions. *Proc Natl Acad Sci USA* 1989; **86**: 8059.
34. Boursot P, Yonekawa H, Bonhomme F. Heteroplasmy in mice with deletion of a large coding region of mitochondrial DNA. *Mol Biol Evol* 1987; **4**: 46.
35. Rotig A, Bessis JL, Romero N *et al.* Maternally inherited duplication of the mitochondrial genome in a syndrome of proximal tubulopathy, diabetes mellitus, and cerebellar ataxia. *Am J Hum Genet* 1992; **50**: 364.
36. Poulton J, Deadman ME, Gardiner RM. Duplications of mitochondrial DNA in mitochondrial myopathy. *Lancet* 1989; **1**: 236.
37. Mita S, Rizzuto R, Moraes CT *et al.* Recombination via flanking direct repeats is a major cause of large-scale deletions of human mitochondrial DNA. *Nucleic Acids Res* 1990; **18**: 561.
38. Schon EA, Rizzuto R, Moraes CT *et al.* A direct repeat is a hotspot for large-scale deletion of human mitochondrial DNA. *Science* 1989; **244**: 346.
39. Puoti G, Carrara F, Sampaolo S *et al.* Identical large scale rearrangement of mitochondrial DNA caused Kearns-Sayre syndrome in a mother and her son. *J Med Genet* 2003; **40**: 858.
40. Pineda M, Ormazabal A, Lopez-Gallardo E *et al.* Cerebral folate deficiency and leukoencephalopathy caused by a mitochondrial DNA deletion. *Ann Neurol* 2006; **59**: 394.
41. Ogasahara S, Yorifuji S, Nishikawa Y *et al.* Improvement of abnormal pyruvate metabolism and cardiac conduction defect with coenzyme Q10 in Kearns-Sayre syndrome. *Neurology* 1985; **35**: 372.

Pearson syndrome

Introduction	398	Treatment	402
Clinical abnormalities	398	References	402
Genetics and pathogenesis	401		

MAJOR PHENOTYPIC EXPRESSION

Anemia or pancytopenia with vacuoles in marrow precursors, exocrine pancreatic insufficiency, hepatic dysfunction, small stature, mitochondrial myopathy, neurologic degeneration, lactic acidemia, and deletions in mitochondrial DNA.

INTRODUCTION

A syndrome was first described in 1979 by Pearson and colleagues [1] from New Haven, Philadelphia, Fort Worth, and Sydney, Australia, in which four unrelated patients had refractory sideroblastic anemia with variable neutropenia and thrombocytopenia, and clinical and pathologic evidence of pancreatic dysfunction. One of these patients later developed Kearns-Sayre syndrome [2]. In 1991, study

of this patient by McShane and colleagues [3] revealed a 4.9-kb deletion in mitochondrial DNA. This 4977-bp deletion was located between nt 8488 and nt 13,460. It was also that most commonly observed in patients with Kearns-Sayre syndrome. The same deletion ([Figure 55.1](#)) had been reported in 1988 by Rotig and colleagues in an infant with Pearson syndrome [4]. Rotig and colleagues [5] have since studied a larger series of nine patients with Pearson syndrome, including one of Pearson's original patients; five had the previously identified 4.9-kb deletion, and four had distinctly different deletions in the same area of the genome. A consistent feature was the occurrence of direct repeats at the boundaries of the deletions [6], providing a possible mechanism for recombinations. Rotig and her colleagues [7] have since found a patient in whom there was an insertion, as well as a deletion in the mitochondrial DNA. In all patients studied, there was heteroplasmy of normal and deleted mitochondrial genomes.

CLINICAL ABNORMALITIES

Patients with this syndrome ([Figures 55.2, 55.3, 55.4, and 55.5](#)) have severe transfusion-dependent anemia [1]. Onset is in the early weeks of life and pallor may be noted in the neonatal period. Anemia is macrocytic and aregenerative. Reticulocyte percentages are low. Hemoglobin F levels may be increased, and the free-erythrocyte protoporphyrin level may be increased [1]. Neutropenia and thrombocytopenia are variable. Either or both may begin concomitantly with the anemia or shortly thereafter, or pancytopenia may

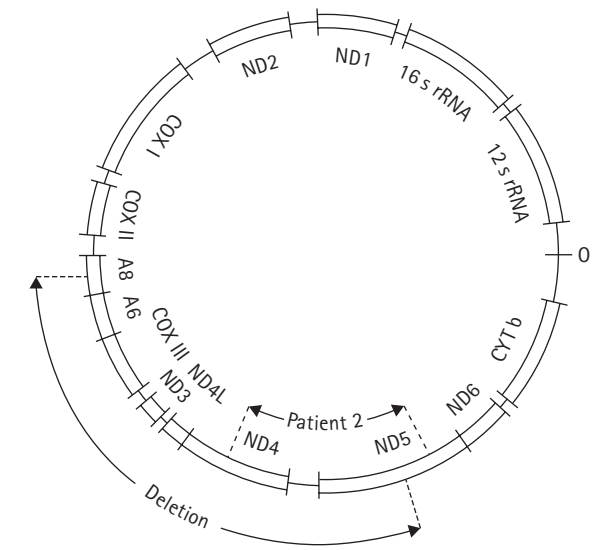


Figure 55.1 The mitochondrial genome and the deletion of 4.9 kb most commonly found in Pearson syndrome.



Figure 55.2 ES: A three-year-old boy with Pearson syndrome. He had failure to thrive, a renal Fanconi syndrome, anemia, and intestinal malabsorption. Renal biopsy revealed interstitial fibrosis and mitochondrial cytopathy. A Broviac port provided venous access for alimentation, as well as transfusion. He also had a gastrostomy.



Figure 55.3 ES: The face was pudgy and hyperpigmented. Despite this Cushingoid appearance, there was no history of steroid treatment. He also had acanthosis nigricans.

become progressively worse. In some, neutropenia may be episodic. Resistance to infection is impaired and death may occur in infancy, from infection such as *E. coli* sepsis. Death prior to three years of age has been reported in 62 percent of patients [7, 8]. Neonatal death has been reported [9]. On the other hand, in patients surviving infancy, the anemia may disappear spontaneously and the hemoglobin stabilize as early as 11 months or two to three years of age. In such a patient, platelet counts may remain low. On the other hand, the anemia may first be evident at 13 months of age [10] with spontaneous recovery seven months later.

Bone marrow at the height of the anemia reveals increased cellularity and there is striking vacuolization of both erythroid and myeloid precursors (Figures 55.6 and 55.7). The vacuoles are not those of fat, glycogen, or lysosomal material, for they were not stained by Giemsa, hematoxylin and eosin, Sudan black, or periodic acid Schiff (PAS). There were increased amounts of hemosiderin in the marrow and ringed sideroblasts (Figure 55.8). Electron microscopy revealed no limiting membranes on the vacuoles. The ringed sideroblast was a nucleated red cell with hemosiderin-laden mitochondria in a perinuclear arrangement. A variety of therapeutic modalities, such as prednisone, B_{12} , folate, and oxymethalone, were without effect.

Patients have varying degrees of pancreatic dysfunction. Some have had steatorrhea and malabsorption, but others have not. Tests of pancreatic abnormality have included decreased response of pancreatic enzymes and bicarbonate



Figure 55.4 KK: A four-month-old patient with Pearson syndrome. He had neonatal onset pancytopenia requiring transfusion. Mitochondrial DNA deletion was documented. Illustrated is the failure to thrive, a consequence of his malabsorption, and the Port-a-Cath for transfusion.

in duodenal aspirates to secretin-pancreozymin, and absence of stool or duodenal tryptic activity. Pancreatic fibrosis was documented in two patients at autopsy [1]. Another patient had chronic diarrhea, but was not evaluated for pancreatic function. Another [11] had increased stool fat; this two-year-old girl also had diabetes and severe renal tubular acidosis. She had polyuria, proteinuria, glycosuria, phosphaturia, and generalized amino aciduria along with systemic acidosis, hypokalemia, and hypophosphatemia. Renal biopsy revealed tubular dilatation, degeneration of



Figure 55.5 KK: He was pale, had lactic acidemia, and mild acidosis.

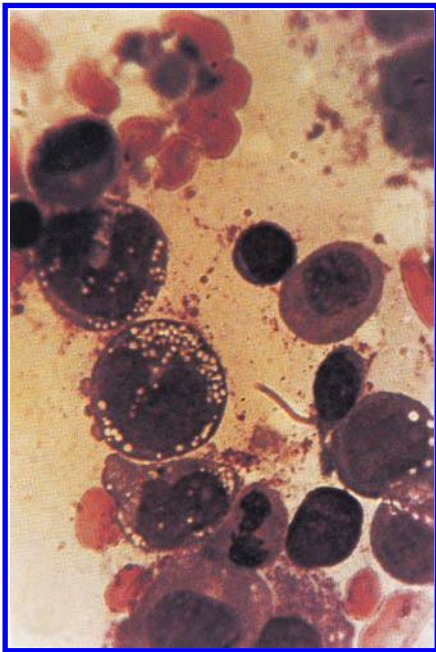


Figure 55.6 Bone marrow aspirate illustrating vacuolation of granulocyte precursors (magnification, $\times 1000$). (Reproduced from the original paper of Pearson *et al.* [1] in the *Journal of Pediatrics* with permission from Elsevier.)

tubular epithelium and giant mitochondria in the proximal tubules. This patient also had hypotonia and had lost the ability to walk; there was muscle wasting and failure to grow. Computed tomography (CT) scan revealed cerebral atrophy. Renal Fanconi syndrome has been observed in others [12]. Hypokalemia and hypercalciuria have been observed in other patients. One patient had renal cysts [13]. Two patients with Pearson syndrome developed insulin-dependent diabetes mellitus [14].

Similarities and differences between this syndrome

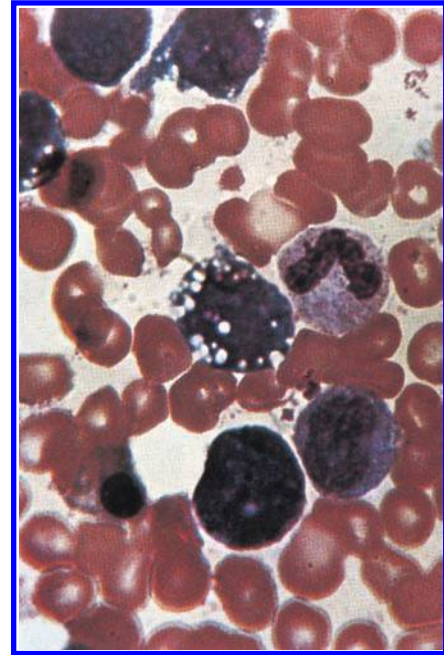


Figure 55.7 Bone marrow also reveals vacuolated erythrocyte precursors (magnification, $\times 1000$). (Reproduced from the original paper of Pearson *et al.* [1] in the *Journal of Pediatrics* with permission from Elsevier.)

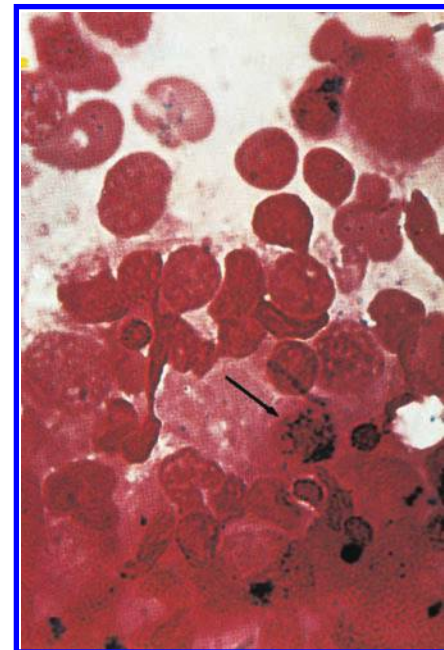


Figure 55.8 Ringed sideroblast is indicated by the arrow. There was hemosiderin throughout the marrow (magnification, $\times 1000$, Prussian blue stain). (Reproduced from the original paper of Pearson *et al.* [1] in the *Journal of Pediatrics* with permission from Elsevier.)

and Schwachman syndrome, in which exocrine pancreatic insufficiency is associated with hematologic disease, have been highlighted [1, 14]. In Schwachman syndrome, the marrow abnormality leads to leukopenia, and the histology is of pancreatic fatty replacement. There is also bony metaphyseal dysplasia. In Pearson syndrome, vacuolation of marrow cells is distinctive; there has also been autopsy evidence of splenic atrophy [1]. In a patient with severe pancytopenia early in life, fibrosis of the thyroid was found at autopsy [15].

Hepatic dysfunction is another feature of Pearson syndrome. In the initial series, the first patient, who died at 26 months, had fatty infiltration of the liver; the second died of hepatic decompensation, and the liver at autopsy showed fat deposits, but no cirrhosis. A number of other patients have died in infancy of hepatic insufficiency [16]. Patients recovering from the marrow dysfunction may develop evidence of hepatic disease manifested by elevated transaminases, lactic dehydrogenase, and hypoprothrombinemia resistant to vitamin K. There may be jaundice.

Cataracts have been observed in Pearson syndrome [17]. Another patient had choroidal dystrophy [18] and hypogonadotropic hypogonadism.

Study of one of the original patients of Pearson *et al.* [1], at the age of 14 years, revealed a very different late-childhood/adolescent phenotype [2]. His hematologic disease began to improve spontaneously at seven months of age, and he had his last transfusion at 11 months, but macrocytosis and slightly decreased neutrophil and platelet counts persisted. Short stature was progressively evident; he was in the 25th percentile at six years and in the fifth at eight years. Over the next three years, growth velocity was 4 cm/year. His 24-hour integrated growth hormone level was judged to be low and he was treated with growth hormone for four months. At 12 years, activity and mental alertness decreased, and he developed a tremor and a stammer. Speech and handwriting deteriorated, and his tremor became increasingly debilitating. Cognitive function was normal and his IQ was 109. Deep tendon reflexes were brisk and there was unsustained clonus. He had a moderate lactic acidosis.

Patients with this disease may also have lactic acidemia in infancy [4, 9]. Some have died in episodes of metabolic acidosis. Magnetic resonance imaging of the brain of such a boy revealed diffuse increase in T_2 -weighted signal in the globus pallidus and pons and the white matter of the cerebral hemispheres, with periventricular sparing. This picture has been noted as having features of Kearns-Sayre syndrome [19]; it is more reminiscent of Leigh syndrome, at least on neuroimaging, and the dominating tremor of the clinical picture could be a different disease because of familial tremor in two generations. The fact that the affected family males were on the maternal side suggests that it is a consequence of the deletion. Other patients have had Leigh-type neuropathology [20, 21].

The patient of McShane and colleagues [3], who

presented in the neonatal period with Pearson-type anemia, later developed a typical Kearns-Sayre picture, with external ophthalmoplegia and pigmentary retinopathy, and mitochondrial myopathy on muscle biopsy, including ragged red fibers. The patient of Nelson *et al.* [22] had Kearns-Sayre syndrome with chronic progressive ophthalmoplegia and myopathy, having had sideroblastic anemia in infancy.

Progressive cardiac dysfunction, affecting predominantly the left ventricle was reported [23] in a five-year-old boy. The authors suggested that this disease should be considered in patients with left ventricular dysfunction and suggestions of mitochondrial disease.

GENETICS AND PATHOGENESIS

The disease is caused by alterations in the mitochondrial genome (Figure 55.1). Heteroplasmy has regularly been observed in affected individuals and the size of the deletion may vary considerably, although the location is always in the same area of the mitochondrial circular genome [3, 5]. Deletions have been identified in every tissue tested, including leukocytes, marrow cells, fibroblasts, and lymphoblasts of an original patient of Pearson [7]. The disorder is often described as sporadic and, in most families, there is no evidence of disease in the mother. However, identical deletions have been reported [10] in a son with Pearson syndrome and his mother with progressive external ophthalmoplegia. A daughter was unaffected. In another family [24], a mother and son had the identical 5355 deletion; he had typical Pearson syndrome, while she had progressive external ophthalmoplegia ptosis and weakness of pharyngeal facial, cervical, and limb muscles. Variability of this sort is consistent with the maternal inheritance pattern of mitochondrial mutations in which there is stochastic segregation of heteroplasmic DNA in the oocyte, and a bottleneck effect in which few of the very many mitochondrial DNAs are selected for the oocyte and new embryo. Certainly, a mother with clinical progressive external ophthalmoplegia is at risk for production of an infant with Pearson syndrome. A clinically normal mother could also have more than one offspring with this syndrome, especially if there were germ-line mosaicism.

The most common mutation is a 4977 base pair deletion (Figure 55.1) from nucleotide 8482 to 13,460 [10]. This is also the most common deletion in Kearns-Sayre syndrome (Chapter 54) [25]. The deletion extends from the ND5 (NADH-CoQ reductase subunit 5) gene to the adenosine triphosphatase (ATPase) subunit 8 gene. There are two origins of mitochondrial DNA replication, with two different origins O_H and O_L for heavy and light chains, respectively, with replication of the former in a clockwise direction and the latter in the reverse. Since deletions usually spare O_H and O_L , there are two areas in which deletions occur most often, including all of

those in Pearson syndrome; that is, in the larger O_H to O_L arc. The 4977-bp deletion is bounded by a pair of 13-bp direct repeats. This is a likely hot-spot for deletion. Rotig and colleagues [5] found different types of direct repeats at the boundaries of five different deletions in the same area in nine patients. There was conservation in the 39 repeated sequences in the deletions and a certain homology between the nucleotide composition of six direct repeats and structures normally involved in replication of mitochondrial DNA and the processing of mitochondrial RNA. These repeats were particularly rich in pyrimidine nucleotides.

The deletions span coding segments for NADH dehydrogenase, cytochrome oxidase, and cytochrome b [26]. This would be expected to lead to disturbance in oxidative phosphorylation and would be consistent with the lactic acidemia observed. Abnormal redox was suggested [4] by a high lactate to pyruvate ratio of 30 (normal, below 20) and 3-hydroxybutyrate to acetoacetate ratio of 4 (normal, below 2). A larger 7767-bp deletion [22] led to deficient polarographic uptake of oxygen in the presence of NAD-linked substrates and enzymatic evidence of deficiency of all of the complexes of the electron transport chain.

Two patients were reported [27] in whom deletions were found along with duplications and deletion dimers. One, considered to have a more attenuated phenotype because she died at ten years of age, had a linear duplication of 25 kb. The other girl, who died at 39 months with a lactic acidemia of 28 mmol/L, had a deletion dimer. Deletion dimers are combinations of two deleted fragments, while duplications are combinations of a normal and deleted fragment. It has been postulated that greater amounts of deletion dimers may correlate with greater severity of clinical manifestations [28]. Duplications may carry a better life expectancy [27], but this reflects experience with only the two patients studied. Duplications appear to be associated with increased recurrence risk as opposed to deletions only [29–32]. The size and percentage of the deletions do not predict the clinical course or severity of disease [27]. Furthermore, there is no minimal region of deletion, the removal of which leads to Pearson or Kearns-Sayre syndrome. However, at least one tRNA must be deleted to cause either of these syndromes [33].

The spontaneous improvement of the anemia with time is of considerable interest. It is consistent with a concept of critical periods in the development of individual tissues. It has been postulated that there might be selective disadvantage with time of rapidly dividing hematopoietic cells containing a high proportion of deleted DNA. The opposite appears to occur in tissues like muscle and brain, where cells turn over more slowly; mutant DNA oxidative function diminishes and mitochondrial encephalomyopathy develops. In these cells, deletions and duplications appear to have selective advantage over wild-type DNA, and, in contrast to mtDNA point mutations, they increase in proportion with time.

The random nature of partitioning of mitochondrial DNA during embryogenesis makes prenatal diagnosis unreliable with either amniocytes or chorionic villus cells. Rearrangements in mtDNA gradually disappear in cultured cells unless uridine is present in the culture medium.

In four patients with Pearson syndrome, 3-methylglutaconic acid excretion was elevated [34]. The authors suggested that the detection of this compound on organic acid analysis of the urine may be a useful marker for the disease.

TREATMENT

Refractory anemia requires repeated transfusion of blood. Erythropoietin is generally ineffective. Thrombocytopenia may require platelet transfusion. Pancreatic extract is useful in the management of the pancreatic insufficiency.

REFERENCES

1. Pearson HA, Lobel JS, Kocoshis SA *et al.* A new syndrome of refractory sideroblastic anemia with vacuolization of marrow precursors and exocrine pancreatic function. *J Pediatr* 1979; **95**: 976.
2. Blaw ME, Mize CE. Juvenile Pearson syndrome. *J Child Neurol* 1990; **5**: 186.
3. McShane MA, Hammans SR, Sweeney M *et al.* Pearson syndrome and mitochondrial encephalopathy in a patient with a deletion of mtDNA. *Am J Hum Genet* 1991; **48**: 39.
4. Rotig A, Colonna M, Blanche S *et al.* Deletion of blood mitochondrial DNA in pancytopenia. *Lancet* 1988; **2**: 567.
5. Rotig A, Cormier V, Koll F *et al.* Site-specific deletions of the mitochondrial genome in the Pearson marrow-pancreas syndrome. *Genomics* 1991; **10**: 502.
6. Kogelnik AM, Lott MT, Brown MD *et al.* A human mitochondrial genome database. Atlanta Center for Molecular Medicine, Emory University School of Medicine. Last accessed December 2004. Available from www.mitomap.org.
7. Rotig A, Cormier V, Blanche S *et al.* Pearson's marrow-pancreas syndrome. A multisystem mitochondrial disorder in infancy. *J Clin Invest* 1990; **86**: 1601.
8. Rötig A, Bourgeron T, Chretien D *et al.* Spectrum of mitochondrial DNA rearrangements in the Pearson marrow-pancreas syndrome. *Hum Mol Genet* 1995; **4**: 1327.
9. Muraki K, Goto Y, Nishinio I *et al.* Severe lactic acidosis and neonatal death in Pearson syndrome. *J Inher Metab Dis* 1997; **20**: 43.
10. Bernes SM, Bacino C, Prezant TR *et al.* Identical mitochondrial DNA deletion in mother with progressive external ophthalmoplegia and son with Pearson marrow-pancreas syndrome. *J Pediatr* 1993; **123**: 598.
11. Majander A, Suomalainen A, Vetterranta K *et al.* Congenital hypoplastic anemia, diabetes and severe renal tubular dysfunction associated with a mitochondrial DNA deletion. *Pediatr Res* 1991; **30**: 327.

12. Niaudet P, Heidet L, Munnich A *et al.* Deletion of the mitochondrial DNA in a case of de Toni-Debre-Fanconi syndrome and Pearson syndrome. *Pediatr Nephrol* 1994; **8**: 164.
13. Gurgey A, Ozalp I, Rotig A *et al.* A case of Pearson syndrome associated with multiple renal cysts. *Pediatr Nephrol* 1996; **10**: 637.
14. Favoreto F, Caprino D, Micalizzi C *et al.* New clinical aspects of Pearson's syndrome: report of three cases. *Haematologica* 1989; **74**: 591.
15. Stoddard RA, McCurnin DC, Shultenover SJ *et al.* Syndrome of refractory sideroblastic anemia with vacuolization of marrow precursors and exocrine pancreatic dysfunction presenting in the neonate. *J Pediatr* 1981; **99**: 259.
16. Comier V, Rotig A, Bonnefont JP *et al.* Pearson's syndrome. Pancytopenia with exocrine pancreatic insufficiency: new mitochondrial disease in the first year of childhood. *Arch Fr Pediatr* 1991; **48**: 171.
17. Cursiefen C, Kuckle M, Scheurlen W, Naumann GO. Bilateral zonular cataract associated with the mitochondrial cytopathy of Pearson syndrome. *Am J Ophthalmol* 1998; **125**: 260.
18. Barrientos A, Casademont J, Genis D *et al.* Sporadic heteroplasmic single 55 kb mitochondrial DNA deletion associated with cerebellar ataxia hypogonadotropic hypogonadism choroidal dystrophy and mitochondrial respiratory chain complex I deficiency. *Hum Mutat* 1997; **10**: 212.
19. Rotig A, Colonna M, Blanche S *et al.* Mitochondrial DNA deletions in Pearson's marrow/pancreas syndrome. *Lancet* 1989; **1**: 902.
20. Santorelli FM, Barmada MA, Pons R *et al.* Leigh-type neuropathology in Pearson syndrome associated with impaired ATP production and a novel mtDNA deletion. *Neurology* 1996; **47**: 1320.
21. Yamadori I, Kurose A, Kobayashi S *et al.* Brain lesions of the Leigh-type distribution associated with a mitochondriopathy of Pearson's syndrome: light and electron microscopic study. *Acta Neuropathol* 1992; **84**: 337.
22. Nelson I, Bonne G, Degoul F *et al.* Kearns-Sayre syndrome with sideroblastic anemia: molecular investigations. *Neuropediatrics* 1992; **23**: 199.
23. Krauch G, Wilichowski E, Schmidt KG *et al.* Pearson marrow-pancreas syndrome with worsening cardiac function caused the pleiotropic rearrangement of mitochondrial DNA. *Am J Med Genet* 2002; **110**: 57.
24. Shanske S, Tang Y, Hirano M *et al.* Identical mitochondrial DNA deletion in a woman with ocular myopathy and in her son with Pearson syndrome. *Am J Hum Genet* 2002; **71**: 679.
25. Wallace DC, Lott MT, Torroni A, Brown MD. Report of the committee on human mitochondrial DNA. *Cytogenet Cell Genet* 1992; **59**: 727.
26. De Vivo DC. The expanding clinical spectrum of mitochondrial diseases. *Brain Dev* 1993; **15**: 1.
27. Jacobs LJAM, Jongbloed RJE, Wijbur FA *et al.* Pearson syndrome and the role of deletion dimers and duplications in the mtDNA. *J Inherit Metab Dis* 2004; **27**: 47.
28. Poulton J, Holt IJ. Mitochondrial DNA: does more lead to less? *Nat Genet* 1994; **8**: 313.
29. Ballinger SW, Shoffner JM, Gebhart S *et al.* Mitochondrial diabetes revisited. *Nat Genet* 1994; **7**: 458.
30. Dunbar DR, Moonie PA, Swingler RJ *et al.* Maternally transmitted partial direct tandem duplication of mitochondrial DNA associated with diabetes mellitus. *Hum Mol Genet* 1993; **2**: 1619.
31. Poulton J, Deadman ME, Bindoff L *et al.* Families of mtDNA re-arrangements can be detected in patients with mtDNA deletions: duplications may be a transient intermediate form. *Hum Mol Genet* 1993; **2**: 23.
32. Rotig A, Bessis JL, Romero N *et al.* Maternally inherited duplication of the mitochondrial genome in a syndrome of proximal tubulopathy, diabetes mellitus, and cerebellar ataxia. *Am J Hum Genet* 1992; **50**: 364.
33. Tang Y, Schon EA, Wilichowski E *et al.* Rearrangements of human mitochondrial DNA (mtDNA): new insights into the regulation of mtDNA copy number and gene expression. *Mol Biol Cell* 2000; **11**: 1471.
34. Gibson KM, Bennett MJ, Mize CE *et al.* 3-Methylglutaconic aciduria associated with Pearson syndrome and respiratory chain defects. *J Pediatr* 1992; **121**: 940.

The mitochondrial DNA depletion syndromes: mitochondrial DNA polymerase deficiency

Introduction	404	Treatment	409
Clinical abnormalities	405	References	410
Genetics and pathogenesis	408		

MAJOR PHENOTYPIC EXPRESSION

Episodic neurologic deterioration and psychomotor regression beginning in the second year of life, with ataxia, encephalopathy, and failure to thrive, progressive to refractory seizures, cortical blindness, stroke-like episodes, acute fulminant hepatic failure, with micronodular cirrhosis, respiratory failure, and coma; fasting hypoglycemia, dicarboxylic aciduria, lactic acidemia, mitochondrial DNA depletion and deficient activity of intramitochondrial DNA polymerase γ .

INTRODUCTION

The first evidence for depletion of mitochondrial DNA in human disease was reported by Moraes and colleagues in 1991 [1]. Three hepatic (newborn, infantile, and toddler) and two nonhepatic, myopathic (infantile and toddler) forms are now recognized. The hepatic forms are more appropriately called hepatocerebral [2]. Both hepatic and myopathic phenotypes have been reported in the products of both consanguineous [3] and nonconsanguineous unions [4]. Each of the three hepatic forms is characterized by episodes of acute liver failure, fasting hypoglycemia, and mitochondrial DNA depletion. The two nonhepatic forms are characterized by nonepisodic myopathy, ragged red fibers, elevated serum creatine kinase (CK), and mitochondrial DNA depletion. An enzymatic diagnosis was established for the hepatic toddler form (Alper syndrome), in which mitochondria are deficient in the enzyme responsible for replicating mitochondrial DNA, DNA polymerase γ [5]. Mutations in the *POLG* gene have now been recognized [6, 7].

In the period since the initial description [1], more than 50 patients have been reported [8]. A diagnosis of mitochondrial DNA depletion may be suspected on the basis of fasting hypoglycemia and liver dysfunction characterized by elevations of gamma glutamyl transferase (GGT), often greater than alanine aminotransferase (ALT)

and aspartate aminotransferase (AST), or by elevated CK and ragged red fibers. It is confirmed by quantitative analysis of mitochondrial DNA in biopsied tissue or, in the case of the hepatic toddler form, by demonstration of mitochondrial DNA polymerase deficiency. In the early infantile-onset hepatocerebral disease, a mutation in the deoxyguanosine kinase gene on chromosome 2p13 has been identified [9]. In addition to this single base deletion in the dGK (*DGUOK*) gene, missense mutations, duplications, and other deletions have been described [10]. In four families with myopathic disease, mutations were defined in the thymidine kinase gene (*TK2*) on chromosome 16q22 [11]. It is clear that there is heterogeneity, because only about 10 percent of patients with similar phenotypes that were tested were found to have mutations in these two deoxynucleoside kinases [10, 12, 13]. Mitochondrial DNA depletion has also been found in patients with encephalomyopathy and defects in the succinyl-CoA synthetase gene (*SUCLA2*) [14]. In addition, mitochondrial DNA depletion has been found in patients with mutations in the twinkle helicase gene (*PEO1*) [15], the adenine nucleotide translocator gene (*ANT1*) [16], inner mitochondrial membrane protein gene (*MPV17*) [16–19], and the B subunit of ribonucleotide reductase (*RRM2B*), as well as the thymidine phosphorylase gene (*TP*) in patients with mitochondrial neurogastrointestinal encephalomyelitis (MNGE). Patients with methylmalonic

acidemia and mitochondrial DNA depletion have mutations in the succinate synthases (*SUCLG1*) [14–19].

CLINICAL ABNORMALITIES

In the neonatal-onset hepatocerebral form of mitochondrial DNA depletion, the infants were small for gestational age (1.9–2.4 kg at term) and developed severe hypoglycemia (to 18 mg/dL, 1.0 mmol/L), and signs of severe liver dysfunction (prothrombin times of 23–30 seconds) in the first day of life. Hepatic size has been increased. Lactic acidemia, metabolic acidosis, and hyperbilirubinemia were inconsistent findings. Some patients had coagulopathy and increased α -fetoprotein. Mitochondrial electron transport studies showed global reductions in complexes I, II/III, and IV activity in biopsied liver, normal succinate dehydrogenase, and a two-fold elevation in carnitine palmitoyl transferase II. Skeletal muscle electron transport activities were normal. Death followed progressive hepatic failure between one and three months of life. Neurologic abnormalities included hypotonia, failure to develop, and horizontal nystagmus [3]. Histopathology of the liver revealed micronodular cirrhosis, cholestasis, glycogen-laden hepatocytes, microvesicular steatosis, and accumulation of iron. Some had giant cell formation. Electron microscopy revealed marked proliferation of pleomorphic mitochondria. In liver, the levels of mitochondrial DNA were significantly depleted (7 percent of normal). Levels of mitochondrial DNA in muscle, kidney, and cultured fibroblasts were normal.

The infantile hepatocerebral form of mitochondrial DNA depletion is characterized by normal intrauterine growth, birth, and delivery [20, 21]. Nevertheless, it may not be different from the early infantile form. The first hint of trouble may be vomiting or feeding difficulty as early as the first month of life. Death may occur as early as seven months. Hypotonia and fasting hypoglycemia often prompt admission to hospital by two to three months of age. One patient was 9 weeks old upon admission [21] and was found to have hepatic dysfunction characterized by elevations in GGT (353 IU/L) greater than AST (188 IU/L) and ALT (123 IU/L); total bilirubin was 5.9 mg/dL (2.6 mg/dL conjugated, 3.3 mg/dL unconjugated). Lactate in the blood was 7.16 mmol/L, and pyruvate was 0.23 mmol/L, yielding an elevated lactate to pyruvate ratio of 31 (normal ≤ 20), consistent with disturbed redox function resulting from a defect in mitochondrial electron transport. The serum concentration of bicarbonate was normal. Cerebrospinal fluid (CSF) concentration of lactate was 5.6 mmol/L. A monitored fast revealed hypoketotic hypoglycemia with 3-hydroxybutyric acid of 0.28 mmol/L and acetoacetate of 0.07 mmol/L. Glucose challenge raised the fasting blood lactate from 3.1 to 6.2 mmol/L. The patient was treated with a low fat (30 percent calories) diet, carnitine, riboflavin, and thiamine, but there was no significant change in blood lactate. Hypotonia persisted and

gross motor development was poor. No other neurologic abnormalities were observed. Hypoglycemia became progressively worse with age, requiring feedings every 2–3 hours. The patient died at seven months of fulminant hepatic failure and coagulopathy. Light microscopy of the liver revealed steatosis. Electron microscopy revealed numerous mitochondria in which cristae were diminished in number or absent. Biochemical studies of liver mitochondrial electron transport complexes I, II/III, and IV revealed global reduction. Succinate dehydrogenase activity was normal. In skeletal muscle, only complex I activity was reduced. Quantitative studies of mitochondrial DNA showed normal levels in kidney, brain, and heart. Skeletal muscle mitochondrial DNA was 50 percent of normal. Mitochondrial DNA in the liver was depleted to 7 percent of normal.

The toddler form of hepatocerebral mitochondrial DNA depletion (Alper syndrome) [5] is associated with an enzymatic deficiency in intramitochondrial levels of DNA polymerase γ , the enzyme responsible for replicating mitochondrial DNA. Figure 56.1 illustrates an affected child at two years of age. This child had an older brother who had died at the age of 21 months during a second acute episode of hypoglycemia, status epilepticus, and acute hepatic failure associated with a ‘Reye-like syndrome’ that followed a febrile illness.

The toddler form of mitochondrial DNA depletion is characterized by normal intrauterine growth, birth, and delivery. Growth, fine motor, gross motor, cognitive, and language development were all normal in the first year of



Figure 56.1 BW: At two years of age, with the hepatic toddler form of mitochondrial DNA depletion, 18 months before his death.

life. At 19 months of age, the patient depicted in [Figure 56.1](#) had an episode of acute truncal ataxia, vomiting, hypoglycemia (34 mg/dL), associated with hypertonia and encephalopathy following a febrile diarrheal illness. The acute hypertonia gradually resolved to mild hypotonia. He recovered from almost all other deficits, leaving only mild residual truncal ataxia. After a second similar episode at 22 months and evidence of expressive language delay, he was evaluated for a possible disorder of fatty acid oxidation. The patient developed hypoglycemia (33 mg/dL) after 15 hours of a monitored fast. Blood acetoacetate was 0.44 mmol/L and 3-hydroxybutyrate was 3.8 mmol/L at the time of hypoglycemia, reflecting intact ketogenesis for age. Urinary organic acids after the fast showed only mild elevations in adipic (70 mmol/mol creatinine), suberic (36 mmol/mol), and sebacic (18 mmol/mol) dicarboxylic acids, and a trans-cinnamoyl glycine of 55 mmol/mol. Both long-chain and medium-chain triglyceride loads resulted in elevated excretion of urinary trans-cinnamoyl glycine (69 and 94 mmol/mol creatinine, respectively) and 3-hydroxydicarboxylic acids. No abnormalities in plasma amino acids were detected. Plasma free carnitine was reduced to 13.6 μ mol/L. Urine carnitine was normal. ALT and AST were slightly elevated at 73 and 124 IU/L, respectively. Lactate in the blood was 2.3 mmol/L. Magnetic resonance imaging (MRI) of the brain was normal. The patient was treated with carnitine, cornstarch, and a low fat diet.

The patient had six more episodes of decompensation over the following two years associated with febrile illnesses. Neurologic manifestations included truncal ataxia, erratic nystagmoid eye movements, focal myoclonic seizures, progressive failure to thrive, and stroke-like episodes. By the age of 38 months, he was unable to walk. At 41 months, he contracted a rotavirus infection that was associated with focal, left-sided epilepsy partialis continua (EPC), transient left hemiparesis, and cortical blindness. Liver enzymes started rising significantly at this time; the GGT was 987, AST 228, and ALT 288 IU/L. Bilirubin was normal. He died at the age of 42 months in liver failure and coma after a 6-week terminal illness; lactic acid concentrations were up to 15 mmol/L.

Autopsy revealed advanced micronodular cirrhosis with regenerative nodules ([Figure 56.2A](#)). Electron microscopy of the liver revealed marked variation in mitochondrial content and morphology in neighboring hepatocytes. Some liver cells showed significant mitochondrial proliferation with a preponderance of tightly packed cristae and occasional mitochondria with concentric cristae ([Figure 56.2B](#)), while other liver cells had apparently normal mitochondria. Microvesicular fat and bile duct proliferation was noted throughout the liver. Skeletal muscle showed mild fiber size variation and mild increase in lipid staining. Electron microscopy of skeletal muscle showed mitochondrial proliferation with numerous pleomorphic forms ([Figure 56.3](#)). In the brain, neuropathologic examination of the frontal cortex showed marked neuronal loss and

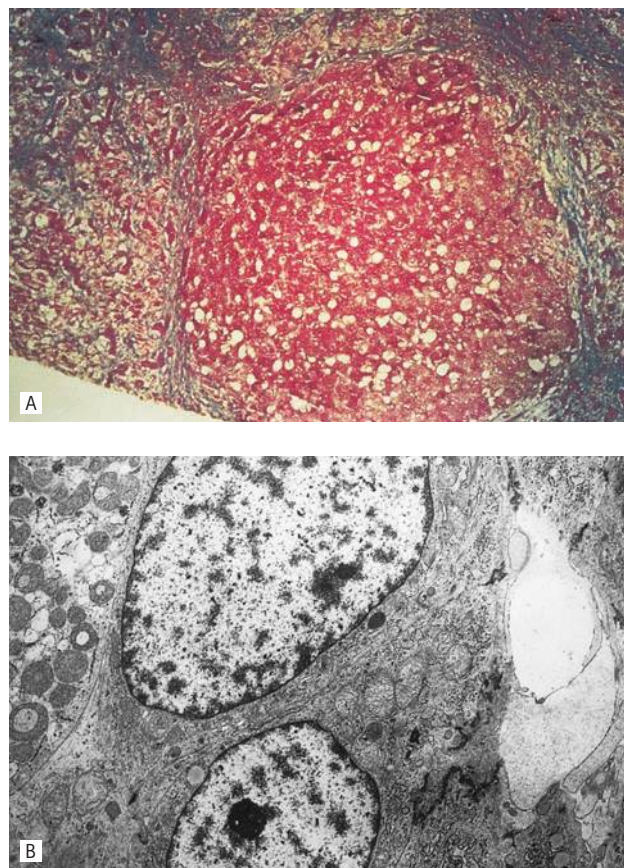


Figure 56.2 Hepatic histology. (A) Gomori-Trichrome. Micronodular cirrhosis, regenerative nodules, microvesicular fat. (B) Electron microscopy. Marked cell-to-cell variation. Mitochondrial proliferation with abnormally packed lamellar and crescentic cristae in some cells, adjoining other cells with normal mitochondrial numbers and cristae.

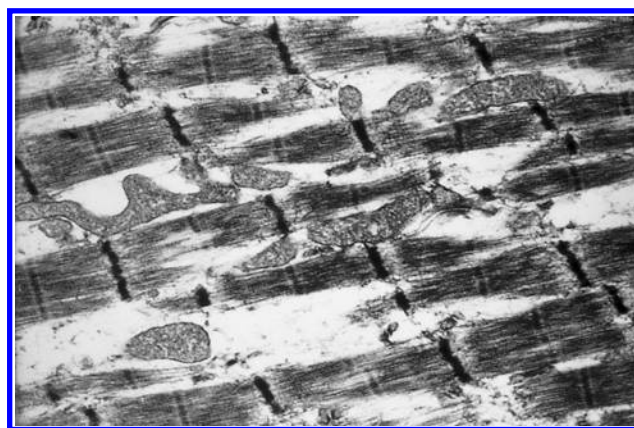


Figure 56.3 Electron microscopy of skeletal muscle. Mitochondrial proliferation and pleomorphic appearance. There were disordered fibers and mildly increased lipid.

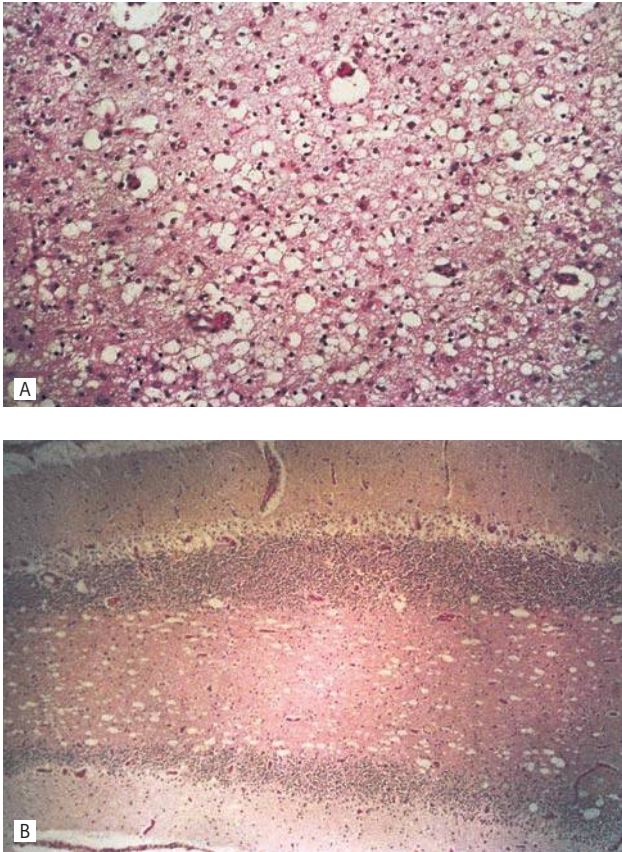


Figure 56.4 Neuropathology. (A) Occipital visual cortex. Fibrous gliosis in layers II, III, and V, with perisomal and perivascular vacuolization. Luxol fast-blue staining of these regions revealed normal myelination. (B) Cerebellum. Total loss of Purkinje cells, prominent gliosis, and sparing of the granular layer. The interfolial white matter displayed prominent spongy vacuolization. There was also focal sclerosis in the cerebellar vermis (not shown).

astrogliosis, and the appearance of Alzheimer type II glia. Subcortical white matter was normal. The primary visual cortex showed gliosis in layers II, III, and V, and perisomal and perivascular vacuolization (Figure 56.4A). Sections through the optic tracts and chiasm showed prominent spongiform vacuolization; however, luxol fast-blue staining of these regions revealed normal myelination. The head of the caudate also showed gliosis. The cerebellar cortex showed a total loss of Purkinje cells, prominent Bergman gliosis and sparing of the granular layer (Figure 56.4B). The interfolial white matter displayed prominent spongy vacuolization. Sections through the vermis showed focal cerebellar sclerosis. The spinal cord also showed spongy vacuolization and gliosis affecting the anterior and posterior spinocerebellar tracts in the lateral columns.

Biochemical studies of the electron transport chain

from skeletal muscle showed global reduction in the activity of complexes I, II/III, and IV. Mitochondrial DNA quantification revealed levels in skeletal muscle that were 30 percent of normal. Assay of purified mitochondria from skeletal muscle and liver revealed a complete absence of activity of mitochondrial DNA polymerase γ .

The nonhepatic infantile form of mitochondrial DNA depletion is characterized by normal intrauterine growth, birth, and delivery [1, 4]. Hypotonia, poor feeding, failure to thrive, and difficulty handling oropharyngeal secretions are noted in the first month of life. Hospital admission is usually prompted by poor motor development, vomiting, dehydration, or respiratory distress by the age of two to four months. At this time, the serum CK may exceed 1000 IU/L. Urinary organic acids are normal. Lactic acidemia may be present during periods of metabolic decompensation, but is not a consistent feature. MRI of the brain reveals delayed myelination. Focal status epilepticus is a late feature. Muscle biopsy reveals abundant ragged red fibers, increased lipid droplets by Oil Red-O staining, and increased glycogen by PAS staining. Histochemical staining for cytochrome oxidase (COX) is absent in fibers. Biochemical studies of electron transport complexes I, II/III, and IV reveal global reduction. Muscle mitochondrial DNA is 2–8 percent of normal.

A fatal neonatal outcome was reported [22] in a patient with myopathic mitochondrial DNA depletion. He developed cyanosis, a weak cry, and generalized hypotonia immediately after birth. Spontaneous movements were diminished, as were reflexes. Ultrasonography of the brain showed periventricular hyperechogenicity and dilated lateral ventricles. EEG was abnormal. There was bilateral renal pyelectasis. There was metabolic acidosis (pH 6.99), lactic acidemia (21 mmol/L), and hyperalaninemia (1.226 mmol/L). He died at 36 hours. Activities of electron transport chain enzymes were markedly reduced in muscle, while in liver there was only a mild reduction of complex I. There was a severe depletion of mitochondrial DNA in muscle while that of the liver was normal.

The nonhepatic toddler form of mitochondrial DNA depletion, which may not be different from the infantile form, is also characterized by normal intrauterine growth, birth, and delivery [1, 4]. In the first year of life, there may be frequent bouts of pneumonia, but without neurologic deficits. Cognitive and motor development are normal in the first year. Between 12 and 16 months, there may be increased stumbling or complete loss of motor milestones. Hyperlordosis and a waddling gait may be present. Patients with DNA depletion in muscle tend to have elevated CK. Serum CK is 500–2000 IU/L. Muscle biopsy reveals type I fiber predominance, abundant ragged red fibers, and a complete absence of COX activity by cytochemical staining. Patients may stop walking by the age of two years and be unable to sit unassisted by two and a half years. Neurodegeneration is progressive to death by respiratory failure in two to four years. Neuropathological examination of cerebrum, cerebellum, brainstem, and spinal cord has

failed to reveal abnormalities. Muscle mitochondrial DNA is 17–34 percent of normal.

Enlarging experience with mutations in *POLG1* suggests that there is a spectrum of clinical phenotypes with presentations from the neonatal period to late adulthood [23]. Severe encephalopathy and hepatic failure represent one extreme. At the other end, are patients with autosomal dominant progressive external ophthalmoplegia (adPEO, MIM 157640) and multiple mitochondrial DNA deletions. Others with multiple deletions have adult-onset cerebellar ataxia. Other dominant kindreds have Parkinson syndrome and premature ovarian failure. Patients with mutations in *RRM1B* had severe hypotonia, nephrocalcinosis and renal tubulopathy, hyporegenerative anemia, and congenital defects [18].

A pharmacogenetic–environmental interaction is susceptibility to hepatic reaction to valproate. In 50 percent of patients with abnormal infantile liver function, the hepatic disease developed within weeks of starting treatment with sodium valproate.

Most adults with *POLG1* mutations developed progressive external ophthalmoplegia (PEO) and myopathy, often in association with ataxia and axonal peripheral neuropathy. Cardiomyopathy occurred in two patients. A majority of severe childhood presentations were in boys.

Two unrelated patients with unusual clinical and biochemical phenotype were reported by Yano and colleagues [24]. Both displayed developmental delay and hypotonia. One had cerebral cortical atrophy and she died in severe metabolic acidosis associated with pneumonia. Both had elevated plasma concentrations of glycine and methylmalonic aciduria (423–520 mmol/mol creatinine). Lactic acid was elevated in blood and urine. Activities of enzymes of the electron transport chain were variably reduced in muscle. The amounts of mitochondrial DNA in muscle were moderately reduced. One of these patients has since been found to have a mutation in the α unit of the succinyl-CoA synthesis gene *SUCLG1*.

Mitochondrial DNA depletion and mutations in mitochondrial DNA *POLG* have now been reported in Leigh syndrome [25].

Among patients with electron transport defects, the enzymatic deficiency may be expressed in liver, even in patients with no sign of hepatic disease [26]. In a series of 31 patients with hepatic enzymatic deficiency, the deficiency was exclusively in liver. These observations provide an argument for liver biopsy in patients who appear to have mitochondrial disease.

Mitochondrial DNA depletion has usually been documented by Southern blot analysis, but the method requires a large amount of DNA, is time-consuming, and susceptible to a number of artifacts. Recent experience with real-time quantitative polymerase chain reaction (PCR) was reported to be more efficient and to have higher sensitivity [27]. An algorithmic approach to the diagnosis of deficiencies of mitochondrial polymerase γ has been developed.

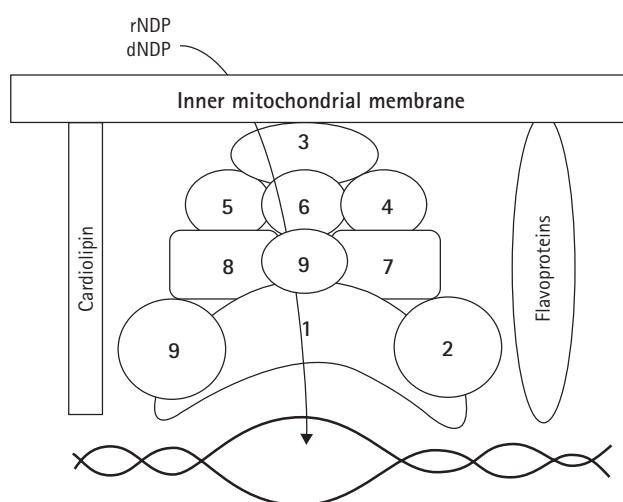


Figure 56.5 Enzymatic components of the mitochondrial DNA replisome: nuclear contributions to mitochondrial DNA replication. All proteins involved in replication are transported to mitochondria via chaperonins (cytoplasmic transport proteins). RNA and DNA are scrolled through the membrane-fixed replisome (comprising about 20 different proteins). Diagram is a model; geometry and composition of the replisome are not yet established. Nuclear gene products known to participate in mitochondrial DNA replication and repair include: 1, DNA Pol γ ($\alpha_2\beta_2$); 2, RNase H (removes primer RNAs); 3, ribonucleotide reductase; 4, nucleoside diphosphate kinase; 5, dihydrofolate reductase; 6, thymidylate synthetase; 7, thymidine kinase; 8, topoisomerase I; 9, DNA ligase I; 10, DNA helicase; 11, ssDNA binding protein; 12, mtTFA; 13, RNase MRP; 14, cytosine deaminase; 15, endonuclease G; 16, mTERF (mitochondrial termination (actor)); 17, TAS-A-BP (termination sequence binding protein); 18, uracil N-DNA glycosylase (UDG, UNG); 19, dUTPase; 20, mitochondrial RNA polymerase; 21, dUMP/dCMO kinase.

GENETICS AND PATHOGENESIS

All of the forms of mitochondrial DNA depletion are transmitted as autosomal recessive disorders [1–3, 26–28].

Molecular defects compatible with the clinical features of these syndromes could include abnormalities in the temporally regulated, tissue-specific expression of the mitochondrial DNA polymerase γ itself, or of one of the other essential components of the mitochondrial DNA replisome (Figure 56.5). One described defect is in the tissue-specific expression of the mitochondrial transcription factor A (mtTFA) required for the production of RNA primers [29]. The cloning of the human mitochondrial DNA polymerase [30] has facilitated molecular dissection. The patient shown in Figure 56.2 had virtually a complete absence of mitochondrial DNA polymerase γ in liver and skeletal muscle.

Human DNA polymerase γ has been characterized as a reverse transcriptase [31, 32]. Two unrelated children with Alpers syndrome were found to have a homozygous

mutation in exon 17 of the POLG locus that led to a glu873 stop. In addition, each was heterozygous for the G1681A mutation in exon 7 that led to an Ala467Thr substitution in the linker region of the protein [6].

The deoxynucleoside kinase defects, deoxyguanosine kinase and thymidine kinase [9, 10], indicate that balanced pools of nucleotides in mitochondria are requisites for mitochondrial DNA replication. These defects provide an additional mechanism for mitochondrial DNA depletion [8]. The single base deletion in the *dGK* gene causes a frame shift and leads to an undetectable enzyme protein [9]. Mutations in this gene and the missense mutations in the *TK2* gene accounted for only 10 percent of the patients with mitochondrial DNA depletion tested [10, 12, 13], so there had to be other causes of this syndrome. In a series of 32 patients with mitochondrial DNA depletion, a molecular cause was defined in 60 percent of patients with myopathy and 80 percent of those with hepatocerebral presentations [19].

Other candidate defects lie in the tissue-specific expression of chaperonins required for accurate trafficking of nuclear gene products, such as the polymerase components of the replisome (Figure 56.5), and components of the respiratory chain into mitochondria.

The mitochondrial genome is a circular double-stranded molecule containing 16,569 nucleotides and coding for 37 genes, including ribosomal RNA and 22 transfer RNAs. Each mitochondrion contains between two and ten copies of the genome.

Pathogenesis follows from mitochondrial DNA depletion. Since 13 protein subunits of complexes I, III, and IV and ATPase of the electron transport chain are encoded by mitochondrial DNA, its depletion would affect the activity of oxidative phosphorylation. The prominent hepatic abnormalities may be explained by the dramatic postnatal developmental changes and mitochondrial adaptation that occur in this organ in the first few months and years of life [33]. Postnatal adaptive changes in skeletal muscle mitochondria occur only later [34]. The existence of nonhepatic (more encephalomyopathic) forms of mitochondrial DNA depletion may reflect early disturbances in the myogenic program that result in either destabilization of the muscle cell membrane, or physical muscle cell breakdown with measurable increases in CK from muscle. Liver, brain, and muscle all 'learn' or undergo adaptive metabolic changes that are shaped by encounters with the postnatal environment. High ratios of NADH to NAD⁺ have been found in the severe deficiencies of complexes I, III, and IV in a patient with mitochondrial DNA depletion [21] and this should decrease mitochondrial β oxidation and provide a mechanism for impaired fatty acid oxidation [35, 36].

Acquired mitochondrial DNA depletion syndromes have recently been described in adults as a complication of the treatment of HIV-1 and hepatitis B virus infections with the reverse transcriptase inhibitors azidothymidine (AZT) [37] and fluoriodoauracil (FIAU) [38]. These

nucleoside analogs are potent inhibitors of the mitochondrial DNA polymerase γ . In experimental animals, the mitochondrial DNA-depleting myopathy produced by AZT is reversible upon discontinuation of the drug. The delayed liver toxicity of FIAU was apparent only after patients had received the drug for 6–8 weeks, and was not reversible upon discontinuation of the drug. Clinical trials of FIAU were suspended in 1994 when six patients died in acute liver failure. Encephalopathy and ataxia were frequent findings in patients with AZT or FIAU toxicity, but causality was difficult to establish because of coexisting disease. Secondary mitochondrial DNA depletion with near-fatal metabolic acidosis and hepatic failure has now been reported [39] in an infant with an HIV infection treated with AZT, didanosine, and zalcitabine. A 79 percent reduction in mitochondrial DNA of muscle reverted to normal after discontinuation of antiviral therapy.

It is interesting to note that the toxicity syndrome of FIAU more closely resembles the hepatic forms of mitochondrial DNA depletion, while the toxicity syndrome of AZT more closely resembles the nonhepatic (more encephalomyopathic) forms. The unexpected biochemical action of these reverse transcriptase inhibitors *in vivo* and the striking clinical overlap between the inborn and acquired forms of mitochondrial DNA depletion stand as clear reminders that our knowledge of developmentally regulated and organ-specific mitochondrial DNA metabolism and replication is far from complete.

TREATMENT

Avoidance of fasting is an important element in management of the hepatic forms of mitochondrial DNA depletion. Uncooked cornstarch at 1 g/kg three times a day, or at least at bedtime, is useful in preventing hypoglycemia. Carnitine (60–100 mg/kg per day) and cofactor therapy including a multivitamin supplemented with coenzyme Q10 (4 mg/kg per day), riboflavin at 50–100 mg twice a day, and niacin at 10–25 mg twice a day appear to be helpful. A diet low in fat (30 percent of calories) appears prudent.

Myoclonic seizures in the hepatic toddler form of the disease have been difficult to control. Trials of clonazepam or amantadine (5 mg/kg per day) should be considered early, if seizures are not controlled by first-line anticonvulsants or ACTH. Valproic acid should be specifically avoided. Lactic acidemia is often a late complication of mitochondrial DNA depletion and may be reduced by treatment with dichloroacetic acid (50 mg/kg per day), but clinical efficiency has not been observed. Thiamine or biotin supplementation has not been successful in reducing lactic acid concentrations.

The multisystem abnormalities in the two later-onset hepatic forms of mitochondrial DNA depletion argue against the potential benefit of liver transplantation.

Treatment of the nonhepatic forms of mitochondrial DNA depletion also includes carnitine and cofactor therapy.

Other supportive measures include good oropharyngeal secretion management, and early gastrostomy tube placement with fundoplication to avoid aspiration and provide adequate nutrition. Management of the rare bouts of metabolic acidosis is with fluids and bicarbonate.

REFERENCES

- Moraes CT, Shanske S, Tritschler H-J *et al.* Mitochondrial DNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases. *Am J Hum Genet* 1991; **48**: 492.
- Feigenbaum A. Answers to missing mtDNA found at last. *Pediatr Res* 2002; **52**: 319.
- Bakker HD, Schotte HR, Dingemans KP *et al.* Depletion of mitochondrial deoxyribonucleic acid in a family with fatal neonatal liver disease. *J Pediatr* 1996; **128**: 683.
- Tritschler HJ, Andreetta F, Moraes CT *et al.* Mitochondrial myopathy of childhood associated with depletion of mitochondrial DNA. *Neurology* 1992; **42**: 209.
- Naviaux RX, Nyhan WL, Barshop BA *et al.* Mitochondrial DNA polymerase γ deficiency and mitochondrial DNA depletion in a child with Alpers syndrome. *Ann Neurol* 1999; **45**: 54.
- Naviaux RK, Nguyen KV. POLG mutations associated with Alpers' syndrome and mitochondrial DNA depletion. *Ann Neurol* 2004; **55**: 706.
- Taanman JW, Rahman S, Pagnamenta AT *et al.* Analysis of mutant DNA polymerase γ in patients with mitochondrial DNA depletion. *Hum Mutat* 2008; **30**: 248.
- Elpeleg O. Inherited mitochondrial DNA depletion. *Pediatr Res* 2003; **54**: 153.
- Mandel H, Szargel R, Labay V *et al.* The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. *Nat Genet* 2001; **29**: 337.
- Salviati L, Sacconi S, Msancuso M *et al.* Mitochondrial DNA depletion and dGK gene mutations. *Ann Neurol* 2002; **52**: 311.
- Saada A, Shaag A, Mandel H *et al.* Mutant mitochondrial thymidine kinase in mitochondrial depletion myopathy. *Nat Genet* 2001; **29**: 342.
- Mancuso M, Salviati L, Sacconi S *et al.* Mitochondrial DNA depletion: mutations in thymidine kinase gene with myopathy and SMA. *Neurology* 2002; **59**: 1197.
- Taanman JW, Kateeb I, Mantau AC *et al.* A novel mutation in the deoxyguanosine kinase gene causing depletion of mitochondrial DNA. *Ann Neurol* 2002; **52**: 237.
- Elpeleg O, Miller C, HersHKovitz E *et al.* Deficiency of the ADP-forming succinyl CoA synthase activity is associated with encephalomyopathy and mitochondrial DNA depletion. *Am J Hum Genet* 2005; **76**: 1081.
- Sarzi E, Goffart S, Seere F *et al.* Twinkle helicase (PEO1) gene mutation causes mitochondrial DNA depletion. *Ann Neurol* 2007; **62**: 579.
- Spinazzola A, Viscomi C, Fernandez-Vizarra E *et al.* MPV17 encodes an inner mitochondrial membrane protein and is mutated in infantile hepatic mitochondrial DNA depletion. *Nat Genet* 2006; **38**: 570.
- Spinazzola A, Santer R, Akman OH. Hepatocerebral form of mitochondrial DNA depletion syndrome: novel MPV17 mutations. *Arch Neurol* 2008; **65**: 1108.
- Spinazzola A, Invernizzi F, Carrara F. Clinical and molecular features of mitochondrial DNA depletion syndromes. *J Inherit Metab Dis* 2008; **32**: 143.
- Carrozzo R, Dionisi-Vici C, Steuerwald U *et al.* SUCLA2 mutations are associated with mild methylmalonic aciduria, Leigh-like encephalomyopathy, dystonia, and deafness. *Brain* 2007; **130**: 862.
- Mazziota MRM, Ricci E, Bertini E *et al.* Fatal infantile liver failure associated with mitochondrial DNA depletion. *J Pediatr* 1992; **121**: 896.
- Maaswinkel-Mooij PD, Van den Bogert C, Sholte HR *et al.* Depletion of mitochondrial DNA in the liver of a patient with lactic acidemia and hypoketotic hypoglycemia. *J Pediatr* 1996; **128**: 679.
- Poggi GM, Lamantea E, Ciani F *et al.* Fatal neonatal outcome in a case of muscular mitochondrial DNA depletion. *J Inherit Metab Dis* 2000; **23**: 755.
- Horvath R, Hudson G, Ferrari G *et al.* Phenotypic spectrum associated with mutations of the mitochondrial polymerase γ gene. *Brain* 2006; **129**: 1674.
- Yano S, Li L, Le TP *et al.* Infantile mitochondrial DNA depletion syndrome associated with methylmalonic aciduria and 3-methylcrotonyl-CoA and propionyl-CoA carboxylase deficiencies in two unrelated patients: a new phenotype of mtDNA depletion syndrome. *J Inherit Metab Dis* 2003; **26**: 481.
- Taanman JW, Rahman S, Pagnamenta AT *et al.* Analysis of mutant DNA polymerase γ in patients with mitochondrial DNA depletion. *Hum Mutat* 2008; **30**: 248.
- Garcia-Cazorla A, De Lonlay P, Rustin P *et al.* Mitochondrial respiratory chain deficiencies expressing the enzymatic deficiency in the hepatic tissue: a study of 31 patients. *J Pediatr* 2006; **149**: 401.
- Chabi B, Mousson de Camaret B, Duborjal H *et al.* Quantification of mitochondrial DNA deletion, depletion, and overreplication: application to diagnosis. *Clin Chem* 2003; **49**: 1309.
- Ricci E, Moraes CT, Serfidei S *et al.* Disorders associated with mitochondrial DNA depletion. *Brain Pathol* 1992; **2**: 141.
- Poulton J, Morten K, Freeman-Emmerson C *et al.* Deficiency of the human mitochondrial transcription factor h-mtTFA in infantile mitochondrial myopathy is associated with mtDNA depletion. *Hum Mol Genet* 1994; **3**: 1763.
- Lecrenier N, Van Der Bruggen P, Foury F. Mitochondrial DNA polymerases from yeast to man: a new family of polymerases. *Gene* 1996; **185**: 147.
- Murakami E, Feng JY, Lee H *et al.* Characterization of a novel reverse transcriptase and other RNA-associated catalytic activities by human DNA polymerase γ : importance in mitochondrial DNA replication. *J Biol Chem* 2003; **278**: 36403.
- Naviaux RK, Markusic D, Barshop BA *et al.* Sensitive assay for mitochondrial DNA polymerase γ . *Clin Chem* 1999; **45**: 1725.
- Valcarce C, Naranterette RM, Encabo P *et al.* Post natal development of rat liver mitochondrial functions. *J Biol Chem* 1988; **263**: 7767.

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34. Sperl W, Sengers RCA, Trijbels JMF *et al.* Postnatal development of pyruvate oxidation in quadriceps muscle of the rat. *Biol Neonate* 1992; **61**: 188.
 35. Latipää PM, Kärki TT, Hiltunen JK, Hassinen IE. Regulation of palmitoylcarnitine oxidation in isolated rat liver mitochondria: role of the redox state of NAD(H). *Biochim Biophys Acta* 1986; **875**: 293.
 36. Jacobs BS, Van der Bogert C, Dacremont G, Wanders RJA. Beta-oxidation of fatty acids in cultured human skin fibroblasts devoid of the capacity for oxidative phosphorylation. *Biochim Biophys Acta* 1994; **1211**: 37.
 37. Arnaudo E, Dalakas M, Shanske S *et al.* Depletion of muscle mitochondrial DNA in AIDS patients with zidovudine-induced myopathy. *Lancet* 1991; **337**: 508.
 38. Marwick C. NIH panel report of 'no flaws' in FIAU trial at variance with FDA report, new probe planned. *J Am Med Assoc* 1994; **272**: 9.
 39. Church JA, Mitchell WG, Gonzalez-Gomez I *et al.* Mitochondrial DNA depletion, near-fatal metabolic acidosis, and liver failure in an HIV-infected child treated with combination antiretroviral therapy. *J Pediatr* 2001; **138**: 748.

DISORDERS OF CARBOHYDRATE METABOLISM

57.	Galactosemia	415
58.	Glycogen storage diseases: introduction	425
59.	Glycogenosis type I – Von Gierke disease	428
60.	Glycogenosis type II/Pompe/lysosomal α -glucosidase deficiency	438
61.	Glycogenosis type III/amylo-1,6-glucosidase (debrancher) deficiency	447

Galactosemia

Introduction	415	Treatment	422
Clinical abnormalities	415	References	422
Genetics and pathogenesis	419		

MAJOR PHENOTYPIC EXPRESSION

Hepatomegaly, jaundice, vomiting, failure to thrive, cataracts, impaired mental development, renal Fanconi syndrome, urinary reducing substance, deficiency of galactose-1-phosphate uridyl transferase.

INTRODUCTION

Galactosemia is an inborn error of carbohydrate metabolism that results from deficiency of galactose-1-phosphate uridyl transferase (EC 2.7.7.12) (Figure 57.1). The disorder was first described in 1935 by Mason and Turner [1]. They found the reducing sugar in the urine and characterized it chemically as galactose. It is now clear that galactosuria may occur also in galactokinase deficiency, and in uridinediphosphate-4-epimerase deficiency. The enzyme deficiency was discovered by Isselbacher and colleagues [2]. The pathway of galactose metabolism had been worked out a few years earlier by Leloir and by Kalckar and their colleagues [3, 4]. The first step in the utilization is its conversion to galactose-1-phosphate (Gal-1-P) [5], which is catalyzed by galactokinase:



Gal-1-P is then converted to glucose-1-phosphate (G-1-P) in a series of two reactions in which uridinediphosphoglucose (UDPG) functions catalytically. The first of these is the uridyl transferase reaction (Figure 57.1), which is followed by the epimerase reaction in which the uridinediphosphogalactose (UDPGal) formed is converted to UDPG [6].

In developed countries, galactosemia is currently detected by programs of neonatal screening in which the transferase enzyme, or galactose content, is assayed in blood. Early diagnosis and compliance with dietary treatment obviate the classic manifestations of the disease.

Nevertheless, we continue to learn from experience, as late complications are recognized in patients who have had early diagnosis and exemplary management. These have included abnormalities in language development [7, 8] and ovarian failure [9–11].

The gene has been assigned to the short arm of chromosome 9, at 9p13 [12]. In classic galactosemia, the classic mutation is a nucleotide change which leads to a p.Q188R change in the enzyme [13]. In patients with Duarte variant detected by newborn screening but not manifesting clinical illness, the mutation is expressed as p.N314D [13].

CLINICAL ABNORMALITIES

Manifestations of galactosemia [1, 14–16] appear usually within days of birth or of the initiation of milk feedings, and they increase in severity in the first months of life. Vomiting and jaundice may develop as early as a few days after milk feedings are begun. Vomiting has rarely been of sufficient severity to lead to surgery for a diagnosis of pyloric stenosis [17]. Anorexia, failure to gain weight or to increase in length, or even weight loss ensue. Hepatomegaly (Figures 57.2 and 57.3) is a constant finding on examination. Parenchymal damage to the liver is progressive to typical Laennec cirrhosis. Patients may have edema, ascites (Figure 57.3), hypoprothrombinemia, and bleeding. Splenomegaly may develop as portal pressure increases. If milk feedings are continued, the disease may be rapidly fatal.

Patients with galactosemia may present first with

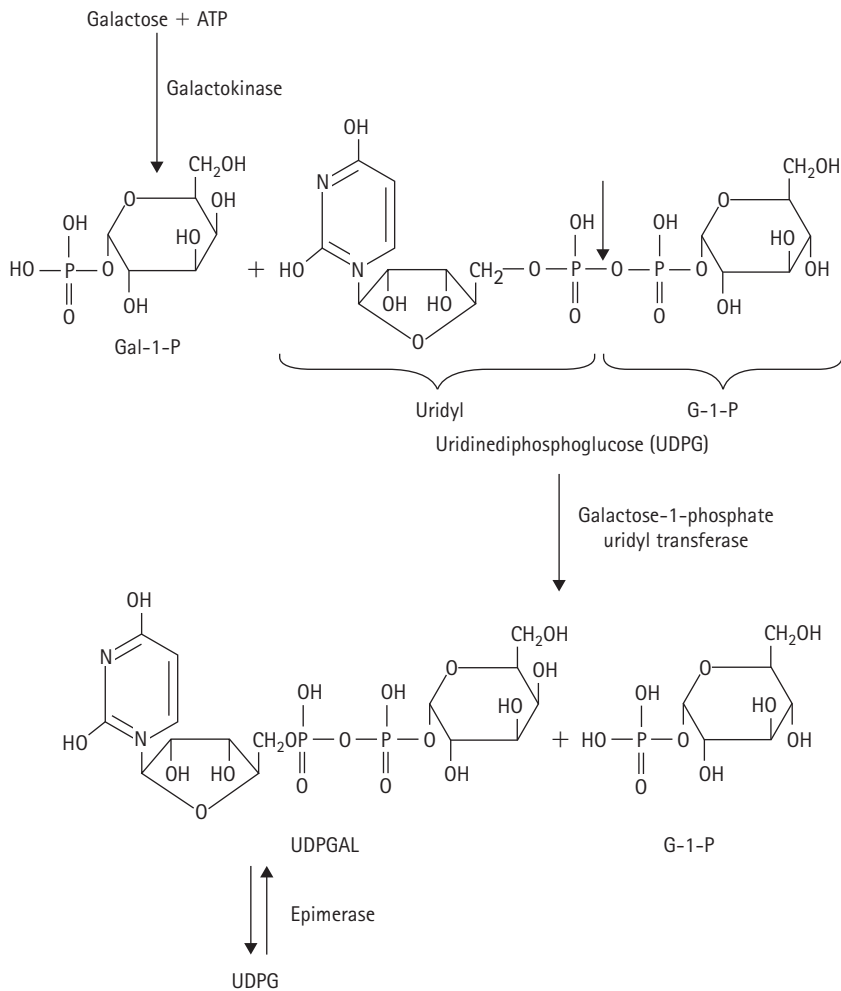


Figure 57.1 Galactose-1-phosphate uridyl transferase, the site of the enzyme defect in patients with galactosemia. The brackets indicate the uridyl and glucose-1-phosphate moieties of uridinediphosphoglucose (UDPG) which have split at the arrow in the uridyl transferase reaction, transferring the uridyl group from G-1-P of UDPG to galactose-1-phosphate (Gal-1-P) to form uridinediphosphogalactose (UDPGAL).



Figure 57.2 Classic presentation of the infant with galactosemia. Hepatomegaly is outlined below the upper line of the rib cage. Failure to thrive is evident in the virtual absence of subcutaneous fat and the folds of loose skin.



Figure 57.3 DS: An infant with galactosemia not diagnosed until 48 days of life. By this time, he had cataracts and failure to thrive. The abdomen was protuberant as a result of hepatomegaly and ascites. He also had edema of the legs and scrotum.



Figure 57.4 Saudi boy with galactosemia. Both lenses were removed because of dense cataracts. He usually wore thick glasses, but they were discarded for the photograph.



Figure 57.5 A: Another Saudi boy with galactosemia and the glasses he wore following surgery for cataracts. His diagnosis was made at one year, at which time he had hepatomegaly 8 cm below the costal margin, bilateral ankle clonus, and patchy white-matter abnormalities on computed tomography (CT), and was unable to sit or stand. Dietary treatment resolved the hepatomegaly. Developmental testing revealed borderline normal intelligence.

sepsis neonatorum. The organism is most commonly *Escherichia coli*. In fact, prior to the advent of neonatal screening programs, the recommendation for the routine testing for galactosemia in all infants with sepsis led to most of the early diagnoses we encountered. A fulminant course of septicemia with early demise has been reported [18]. Complications of sepsis, such as osteomyelitis and meningitis, have also been observed. One patient developed gangrene of the toes bilaterally and of the dorsum of one foot [19]. Granulocyte function may be impaired [20, 21].

The development of lenticular cataracts is a characteristic feature of the disease and occurs in infants who have received milk for 3–4 weeks (Figures 57.4 and 57.5). Early cataracts may be visible by slit lamp examination, as early as after a few days.

Impaired mental development is an important manifestation of the disease. It is most severe in patients who are not diagnosed or treated until a number of months has elapsed. Untreated or poorly compliant patients are often hyperactive. In a series of 41 patients from before the advent of neonatal screening, three had severely impaired mental development, seven had IQ levels between 70 and 84, and 29 had IQs greater than 85 [22]. This experience, of course, reflects some siblings of patients in whom treatment from birth was possible. In another series of 44 [23], eight had IQ levels below 70; ten had levels from 71 to 89; and the rest had normal IQs, but lower than those of unaffected siblings. A relationship to compliance with diet was evident in an average IQ of 84 in 32 highly compliant patients and 77 in 22 poorly compliant patients [15].

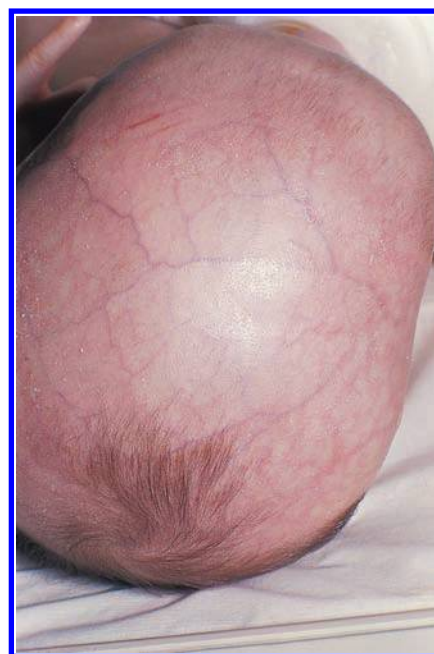


Figure 57.6 DS: Pseudotumor cerebri. The head circumference was increased and the fontanel bulging; the venous pattern was prominent.

Pseudotumor cerebri has been observed in a number of patients with galactosemia (Figure 57.6) [24]. It may be recognized by bulging of the anterior fontanel or by computed tomography (CT) scan [25] or magnetic resonance imaging (MRI) in which cerebral edema is evident. The occurrence of pseudotumor cerebri in patients with galactokinase deficiency [26] indicates that it is the accumulation of galactitol that causes this feature of the disease.

Renal abnormalities usually are first detected in the laboratory by the analysis of the blood and urine. Some patients have had frequency of urination. The picture is that of the renal Fanconi syndrome, in which there is renal tubular glycosuria, generalized aminoaciduria, and proteinuria, and systemically there is hyperchloremic acidosis. The glycosuria of the Fanconi syndrome may cause the galactosuria to be missed. In the past, the initial clinical suspicion of galactose has come from the presence of reducing substance in a sample of urine that tests negative for glucose with a glucose oxidase, but once renal dysfunction develops, both tests would be positive. In any case, most clinical laboratories now test for urinary sugar with glucose oxidase. Tests for reducing substance have become the province of the biochemical genetics laboratory. At times of acute illness, there may be hypoglycemia. In young infants, hematological examination may reveal an erythroblastotic picture.

In general, long-term follow-up study on galactosemic individuals [27] showed that when diagnosis is early and compliance with therapy is good, levels of patient IQ have been normal. Experience with differing times of initiation of therapy, including sibling pairs in whom therapy could be started on the first day of life, provided a trend that indicated the earlier the diagnosis, the higher the IQ. A number of children have had problems in school, so that the performance may not be as good as the IQ would suggest, but overall results have been excellent.

While dietary therapy for galactosemia has effectively eliminated the acute toxicity syndrome of classical galactosemia, long-term complications have become evident as significant problems, even under ideal conditions of management and patient compliance.

Ovarian failure has been recognized in female patients [9–11]. It may present as either primary or secondary amenorrhea with hypergonadotrophic hypogonadism. This is seen in 75–96 percent of female patients by the age of 30 years. The incidence of ovarian failure is unrelated to the age at diagnosis or the degree of dietary control. The mechanism remains enigmatic. Impaired oocyte maturation and accelerated atresia have both been reported. One patient had normal ovaries at laparoscopy at seven years of age and streak ovaries ten years later, suggesting a time-dependent effect. Pregnancies have occurred in female patients with classical galactosemia, although they are very rare. One patient, who successfully delivered, developed ovarian failure later. Many have low levels of estradiol and elevated levels of gonadotropins.

Diminished or absent ovarian tissue may be revealed by ultrasonography. Evidence of hypergonadotrophic hypogonadism has also been found in prepubertal girls [10]. The effect on the ovary is clearly a toxic one that takes a variable period of time to develop.

In the female patients with hypogonadism, thyroid hormone levels were normal, but low concentrations of thyroxine have been reported in two galactosemic infants in whom levels of T_4 became rapidly normal when a galactose-free diet was instituted [28]. Testing for thyroid function in such an infant would be suggested by the presence of jaundice; the finding of a low T_4 might lead away from the diagnosis of galactosemia. This problem should be less frequently encountered where there are programs of neonatal screening, because currently virtually all infants are tested for galactosemia and hypothyroidism before the development of symptoms.

The second major later complication of classical galactosemia is delayed speech and language [7, 8, 29, 30]. Onset of speech has been delayed and there have been problems of articulation and word retrieval. Most children with galactosemia have delayed language development associated with a verbal dyspraxia, but it is often overcome with time. This complication too appears to be unrelated to the time of diagnosis or the level of compliance as assessed by erythrocyte levels of galactose-1-phosphate. Some of those individuals had never received milk and exemplary galactose-1-phosphate concentrations had been maintained.

Cognitive development is the most important long-term issue in this disease. Impaired mental development is severe in patients who are diagnosed and treated late. Prior to the advent of neonatal screening, 11 of 85 patients had IQ levels below 70. An average IQ of 84 was seen in 32 highly compliant patients and 77 in 22 poorly compliant patients. Early information on the development of patients diagnosed early and compliant with therapy was optimistic [27]. By 1972, data from the largest experience in the United States suggested that such patients had normal levels of IQ, and it appeared that the earlier diagnosis, the higher the IQ.

However, more recent experience has led to a much more pessimistic prognosis. The results of a retrospective questionnaire survey of 298 patients from the United States and Europe on whom IQ data were available [30] indicated that 45 percent of those at least six years old were developmentally delayed. This survey provided the first evidence of a definite decline in IQ with age; furthermore, the decline in females was significantly greater than in males. In a more recent retrospective study of 134 galactosemic patients in Germany, there was also evidence of decline in IQ with age in that four of 34 patients less than six years of age had IQs less than 85, ten of 18 between seven and 12 years of age, and 20 of 24 older than 12 years had such levels. A best fit regression line suggested a mean loss in cognitive performance of two IQ points per year; 40 points in 20 years. Of course, most of these patients, especially the older ones, antedated nationwide neonatal screening

in Germany, and in the earlier international study, 270 patients had clinical symptoms prior to diagnosis and treatment. Data were not specifically set out in either study for patients diagnosed presymptomatically and managed carefully. Nevertheless, decline with age in the earlier study was even shown in individuals tested at different ages. In addition, there was evidence in both studies of microcephaly and specific neurological manifestations, such as progressive ataxia and tremor.

MRI of the brain has revealed a substantial number of patients with cerebral atrophy [31]. White matter abnormalities occurred in 95 percent (52 of 55) over one year of age and persisted in follow-up studies one to four years later. In addition, many patients, even with normal IQs, have had problems with behavior and school performance.

A curious syndrome of neurologic abnormality was reported [32] in siblings with galactosemia. Both had impaired mental development, hypotonia, and a coarse tremor. Ataxia developed, and neurologic tests of cerebellar function were abnormal. Dietary control of galactose intake was excellent and documented by determination of levels of Gal-1-P. These manifestations are reminiscent of chicks given lethal doses of galactose [33]. On the other hand, there is a possibility that the siblings each received two rare, recessive, possibly linked genes, even in the absence of consanguinity.

An interesting observation was the development of galactose toxicity despite continuation of a lactose-free diet in a homozygous woman, during lactation. The development of cataracts has been reported in lactating women, even heterozygotes [34, 35].

GENETICS AND PATHOGENESIS

Galactosemia is inherited as an autosomal recessive trait [36]. The enzyme defect in galactosemia is in the uridyl transferase enzyme (Figure 57.1) [2]. The abnormality can be detected in the erythrocyte. Cord blood is a useful source for early diagnosis. The defect can also be detected in cultured fibroblasts and amniotic cells, leukocytes, and liver [37]. The other enzymes of galactose metabolism are normal.

The enzyme is a dimer in which each identical subunit has a molecular weight of 44 kDa [38]. In patients with classic galactosemia, the activity of the enzyme is virtually completely absent [37, 39]. In heterozygotes, the levels are intermediate between patients and normals [39]. The Beutler assay [40], in which the glucose-1-phosphate product is converted to glucose-6-phosphate and the reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH) formed is determined fluorimetrically, has been widely adopted for purposes of neonatal screening [41].

The demonstration of the enzyme defect in galactosemia was the first evidence for human variation at the Gal-1-P uridyl transferase locus. There has since

been evidence of abundant variation, and the first to be discovered was the Duarte variant [42]. This enzyme has a distinct electrophoretic pattern of rapid migration [43], and its activity is about 50 percent of the normal enzyme. It produces no clinical manifestations. A number of other electrophoretic variants has been described. The Los Angeles variant, a rapidly moving enzyme with three bands, has normal or greater than normal activity [44]. The others all have less than normal activity, and in some there may be clinical manifestations. A black variant may have no erythrocytic activity, but has about 10 percent of normal activity in liver and intestine [45]. These patients may have some neonatal symptoms. The Munster variant is associated with a classic galactosemic picture [46]. Gel electrophoresis [47] and isoelectric focusing [48, 49] have been employed to distinguish the variants, but they are being supplanted by molecular methods of determination of mutation.

It has become apparent that compounds in which an individual is heterozygous for two distinct variants are relatively frequent occurrences. Compounds in which one gene is the galactosemic (G) and one the Duarte (D) variant have been the most commonly encountered [50], especially in programs of neonatal screening for galactosemia [50–52]. Most individuals with this phenotype have no clinical manifestations, but transient jaundice, lethargy, and hepatomegaly have been reported [51] in an infant whose mother had sepsis prior to the delivery; and others have displayed biochemical evidence of accumulation of galactose and Gal-1-P in the blood. Transferase levels may be very low early in life [51] and galactose tolerance tests have yielded evidence of diminished ability to metabolize ingested galactose [52].

Polymorphism complicates the determination of heterozygosity, but family study may elucidate the problem. The mean enzyme activity for heterozygotes for the galactosemia variant (GN) approximates half that of normal (N) individuals, and this is the level observed in Duarte homozygotes (DD). However, since heterozygotes for the Duarte variant have about 75 percent of normal activity, the study of parents should clarify the issue. Prenatal diagnosis has been carried out by assay of the enzyme in cultured amniocytes [53] and chorionic villus material, and by the direct measurement by gas chromatography-mass spectrometry (GCMS) of galactitol in the amniotic fluid [54].

Programs of neonatal screening for galactosemia have indicated a frequency of one in 55,000 [55]. The galactosemia–Duarte (GD) compound occurs in about one in 3000–4000 [55]. In one year's experience in California, the frequency of galactosemia was one in 123,000 and that of the compound was one in 38,000, while the three-year experience with the program yielded an incidence of galactosemia of one in 86,000. In Massachusetts, screening of six million neonates yielded a figure of one in 62,000.

In classic galactosemia and in the Duarte variant, there are immunoreactive transferase proteins (cross-reacting

Table 57.1 Protocol for galactosemia

When newborn screening reports a low Gal-1-P uridyl transferase		Remove galactose/lactose from diet and prescribe a soy or other lactose/galactose-free formula. Breastfeeding is stopped
		Obtain blood for Gal-1-P and uridyl transferase mutations
Diagnosis of DD, DG, or GG	DD diagnosis	Return infant to normal diet, no follow up required
	DG diagnosis	Continue diet until infant is 8–12 months old
		Check Gal-1-P
		Begin full lactose/galactose diet for 2 weeks
		Recheck Gal-1-P
		If Gal-1-P is within normal range, continue unrestricted diet
		If Gal-1-P is elevated, then return to lactose/galactose restricted diet
	GG diagnosis	Continue lactose/galactose restricted diet
		Obtain blood for Gal-1-P, LFT, bilirubin, and albumin
		Refer to ophthalmologist
		Monitor monthly in metabolic clinic until four months old, checking Gal-1-P levels every 2 weeks
		If Gal-1-P levels indicate good control, change to monthly Gal-1-P levels and continue monthly clinic visits
		Second year of life: monitor every three months ^a
		2–5 years old: monitor every six months ^a
		6 years and older: monitor yearly ^a
		Evaluate language development at preschool age
		Evaluate ovarian function of teenage girls
		Encourage regular eye examinations
		Developmental assessment at 4, 8, 14, and 18 years

^aMay vary depending on control.

material (CRM)) and the size and structure of these proteins are similar to those of the normal enzyme [56].

The transferase gene has been localized to chromosome 9p13 [12, 57–59] and the cDNA has been cloned from human fibroblasts [60]. The gene is small; 11 exons and 10 introns are found in 3.9 kb. A number of mutations have been identified (Table 57.1) [61–65]. In classic galactosemia, an A to G missense mutation codes for a change from glutamine at position 188 to arginine. In the Duarte variant, an A to G mutation in exon 10 has changed an asparagine to an aspartic acid at position 314 near the carboxy terminus. The most common African mutation is p.S135L. The A to G mutation in the p.Q188R variant introduces a site of cleavage by the restriction endonuclease *HpaII*, which permits family studies and population screening. In addition to Q188R, p.K285N is also common in Caucasians; these two were found in more than 70 percent of alleles [13]. A 4-bp deletion in the 5′ region of the *GALT* gene has been found to be linked to the Duarte allele and to yield reduced activity of the enzyme [66]. The DAT+314 as opposed to N is common in nonhuman species, such as chimpanzee, macaque, and mouse, suggesting that it is the ancestral gene [66].

An interesting biochemical phenotype was found in a family in which the proband had classical galactosemia [67]. He had inherited two mutations in cis from his father, p.N324D and E204K. From the mother, he received a mutation in the splice site acceptor of intron C. Enzyme activity in the father was nearly normal. An asymptomatic sister had compound heterozygosity for three mutations, p.E293K–N324D/N324D. Her erythrocyte enzyme activity was normal. It was speculated that the codons for E203K and N314D led to intra-allelic complementation in cis, but in fact the result is in keeping with what was later established for the effect of the p.N314D Duarte mutation.

The ideal approach to diagnosis of galactosemia is through routine neonatal screening. A protocol for the screening and management of galactosemia is given in Table 57.2. The assay in the United States is for the activity of galactose-1-phosphate uridyltransferase in dried blood on filter paper. In some countries, the assay is for galactose, and this will also detect galactokinase deficiency. The test for galactose will also be positive in patients with congenital shunts from portal to systemic vessels [68]. A positive screening test is confirmed by quantification of activity in freshly obtained erythrocytes in the fluorimetric assay

Table 57.2 Mutations associated with galactosemia

Codon and amino acids substitution ^a	Nucleotide change	Phenotype	Prevalence in classic galactosemia		
			Caucasian	Hispanic	African American
Q188R	CAG→CGG	G	62%	58%	12%
V44M	GTG→ATG	G			
S135L	TCG→TTG	G	0%		48%
M142L	ATG→AAG	G			
R148W	CGG→TGG	G			
L195P	CTG→CGG	G			
R231H	CGT→CAT	G			
H319Q	CAC→CAA	G			
R333W	CGG→TGG	G			
N314D	AAC→GAC	Duarte	5.9% of non-galactosemia controls		

^aWithin galactose-1-phosphate uridyl transferase (GALT).

for NADPH formed along with glucose-6-phosphate from the glucose-1-phosphate product. In classic galactosemia, the activity approximates zero. Variants with greater activity than this can be elucidated by electrophoresis or by mutational analysis. It is important for clinicians to recognize the early clinical manifestation of galactosemia and its infectious complications, because some developed countries have given up neonatal screening for this disease, and even in screened infants classical disease can develop before the results of screening are known. The screening assay is followed by quantification of activity in freshly obtained erythrocytes.

In populations in which screening programs are not available, the diagnosis of infants with early symptoms is still initiated by the finding of galactose in urine. It is important to emphasize that testing of urine with glucose oxidase (Clinistix, Tes-tape) will not detect galactose; this is a strong argument for continued use of the older methods for the screening of urine for reducing substance (Benedict or Fehling test, Clinitest). We have also recognized galactosemia by finding galactose on GCMS of the urine sent for organic acid analysis. It is also true that the excretion of galactose in the urine depends on dietary intake of lactose; in an acutely ill patient admitted to hospital and treated with parenteral fluid therapy, the disease may not be recognizable by testing the urine because he or she has not received galactose for 24 to 48 hours.

Characterization of the reducing substance found in a urine sample can be done in a number of ways. It is usually done by paper chromatography [69]. Testing with paper infiltrated with galactose oxidase provides for an effective screening procedure [70]. Of course, sugar in urine of an infant who tests positive for reducing substance and negative for glucose oxidase is galactose until proved otherwise and indicates direct assay of the enzyme. In

patients with normal activity of the uridyl transferase, assays are performed for galactokinase and epimerase.

The structure of galactose is identical to that of glucose, except for the position of the hydroxyl on carbon 4. Lactose, the principal sugar of mammalian milks, is the predominant dietary source of galactose. It is a disaccharide in which glucose and galactose are linked in an α -1,4-glucosidic bond in which an oxygen bridge connects carbon 1 of galactose and carbon 4 of glucose.

The pathogenesis of most of the clinical manifestations of galactosemia is the accumulation of Gal-1-P in tissues [71]. Among the best evidence for this is the observation that therapeutic measures that result in reduction of intracellular concentrations of Gal-1-P lead to prevention or disappearance of symptoms. It is clear that the manifestations of galactosemia do not occur in galactokinase deficiency, in which disease hepatic, renal, and cerebral damage is unknown. Thus, impaired mental development is not due to galactose itself. Cataracts and pseudotumor cerebri occur in patients with galactokinase deficiency [26, 72, 73], and these complications are due to galactitol. This byproduct of galactose accumulation occurs by its reduction at carbon-1 and is present in urine and tissues. In the lens, galactitol causes osmotic swelling and disruption of fibers. Osmotic swelling is also the mechanism of production of cerebral edema. In addition, cataracts that result from galactose treatment of rats are prevented by sorbinil, which inhibits aldose reductase, the enzyme that catalyzes the conversion of galactose to galactitol [74]. Galactitol has been demonstrated *in vivo* by proton magnetic resonance spectroscopy in the brain of an encephalopathic infant with galactosemia [75].

The pathogenesises of the later appearing dyspraxic speech and ovarian failure, as well as the potential loss of late cognitive function, are not clear. Low concentrations

of UDPGal have been proposed as a mechanism [76]. Information on mutations has indicated that the Q188R/Q188R genotype is a significant predictor of developmental verbal dyspraxia in patients with good metabolic control as indicated by erythrocyte Gal-1-P levels less than 3.2 mg/dL [77].

The possibility of epigenetic effects has been proposed [78] in studies of four patients with quite different cognitive results. Three had the p.Q188R mutation and one who had severely impaired mental development had the usually milder p.S135L mutation. A gene expression profile identified aberrations in cell survival pathways, such as mitogen activated protein kinase (MAPK). Studies of glycosylation of N-linked glycoproteins revealed persistent aberrant glycosylation.

TREATMENT

The treatment for galactosemia is exclusion of galactose from the diet [79]. This is accomplished by the elimination of milk and its products. The mainstay of the diet for an infant is the substitution of casein hydrolysate or a soybean preparation for milk formulas. Education of the parents and of the child as he or she grows older on the galactose content of foods is important. A list of foods has been published that is useful in management [80]. The determination of the Gal-1-P content of erythrocytes is employed in monitoring adherence to the diet [81], and acceptable levels have been set at 4 mg/dL (150 mmol/L). When this is not available, the serum bilirubin and the transaminase levels tend to be employed.

Experience with early treatment supports the concept that effective treatment instituted in the first weeks of life can prevent most of the classic manifestations of the disease. At the other end of the scale, impaired mental development, once established, is irreversible, and if the diagnosis is delayed, some damage to the brain is inevitable. There may be abnormalities of visual perception, behavior problems, or convulsions. Cataracts are reversible if treatment is started within the first three months of life. Hepatic and renal manifestations of the disease are reversible. Late manifestations of language development and ovarian failure are not prevented by otherwise effective treatment. The results of treatment on long-term cognitive function are controversial, but it is clear that prognosis is not as good as it was once thought.

A recent report of long-term follow up of 28 patients treated for classic galactosemia showed mean below average function across a broad spectrum of cognitive measures [82]. However, there was a wide range, and some individuals had average or above average success.

Management of infants detected by newborn screening and found to have DG variants has been controversial, especially in women committed to breastfeeding their infants. In a Philadelphia report [83], 17 DG infants were treated with a low lactose diet for the first year and

11 received a regular diet. While there were significant differences in urine galactitol and red blood cell Gal-1-P throughout the first year, there was no variation in differences in developmental outcomes, including those of IQ and language, but the year of life for this assessment ranged from one to six years, and there were significant differences in adaptive scores.

Monitoring biochemical correlates of treatment in DG patients receiving a standard diet revealed erythrocytes galactose-1-phosphate concentrations in the reference range [84]. However, plasma concentrations of galactose and galactitol were about twice that of control. These elevations showed no relationship to developmental or clinical outcomes.

REFERENCES

1. Mason HH, Turner ME. Chronic galactosemia. *Am J Dis Child* 1935; **50**: 539.
2. Isselbacher KJ, Anderson EP, Kurahashi K, Kalckar HM. Congenital galactosemia a single enzymatic block in galactose metabolism. *Science* 1956; **123**: 635.
3. Leloir LF. The enzymatic transformation of uridine diphosphate glucose into a galactose derivative. *Arch Biochem Biophys* 1951; **33**: 186.
4. Kalckar HM, Braganca B, Munch-Petersen A. Uridyl transferase and the formation of uridine diphosphate galactose. *Nature* 1953; **172**: 1038.
5. Kosterlitz HW. The structure of the galactose-1-phosphate present in the liver during galactose assimilation. *Biochem J* 1943; **37**: 318.
6. Gitzelmann R, Steinmann B. Uridine diphosphate galactose 4-epimerase deficiency. II. Clinical follow-up biochemical studies and family investigation. *Helv Paediatr Acta* 1973; **28**: 497.
7. Waisbren SE, Norman TR, Schnell RR, Levy HL. Speech and language deficits in early treated children with galactosemia. *J Pediatr* 1983; **102**: 75.
8. Nelson CD, Waggoner DD, Donnell GN *et al*. Verbal dyspraxia in treated galactosemia. *Pediatrics* 1991; **88**: 346.
9. Kaufman FR, Kogut MD, Donnell GN *et al*. Hypergonadotropic hypogonadism in female patients with galactosemia. *N Engl J Med* 1981; **304**: 494.
10. Steinmann B, Gitzelmann R, Zachmann M. Hypergonadotropic hypogonadism found already in pre-pubertal girls but only in adult males. *Pediatr Res* 1981; **15**: 1182.
11. Gibson JB. Gonadal function in galactosemics and galactose intoxicated animals. *Eur J Pediatr* 1995; **154**(Suppl. 2): S14.
12. Shih LY, Suslak L, Rosin I *et al*. Gene dosage studies supporting localization of the structural gene for galactose-1-phosphate uridyl transferase (GALT) to chromosome 9. *Am J Med Genet* 1984; **19**: 539.
13. Elsas LJ II, Lai K. The molecular biology of galactosemia. *Genet Med* 1998; **1**: 40.
14. Donnell GN, Bergren WR, Cleland RS. Galactosemia. *Pediatr Clin North Am* 1960; **7**: 315.

15. Holzel A, Komrower GM, Schwarz V. Galactosemia. *Am J Med* 1957; **22**: 703.
16. Komrower GM, Lee DH. Long term follow-up of galactosemia. *Arch Dis Child* 1970; **45**: 367.
17. Nyhan WL. Introduction and general features. In: Nyhan WL (ed.). *Abnormalities in Amino Acid Metabolism in Clinical Medicine*. Norwalk, CT: Appleton Century Crofts, 1984: 5.
18. Levy HL, Sepe SJ, Shih VE et al. Sepsis due to *Escherichia coli* in neonates with galactosemia. *N Engl J Med* 1977; **297**: 823.
19. Collip PJ, Donnell GN. Galactosemia presenting with gangrene. *J Pediatr* 1959; **54**: 363.
20. Kobayashi R, Blum P, Gard S et al. Granulocyte function in patients with galactose-1-phosphate uridytransferase deficiency (galactosemia). *Clin Res* 1980; **28**: 109A.
21. Litchfield WJ, Wells WW. Effects of galactose on free radical reactions of polymorphonuclear leukocytes. *Arch Biochem Biophys* 1978; **188**: 26.
22. Donnell GN, Koch R, Bergren WR. Observations on results of management of galactosemic patients. In: Hsia DYY (ed.). *Galactosemia*. Springfield, IL: Charles C Thomas, 1969: 247.
23. Nadler HL, Inouye T, Hsia DYY. Clinical galactosemia: a study of fifty-five cases. In: Hsia DYY (ed.). *Galactosemia*. Springfield, IL: Charles C Thomas, 1969: 127.
24. Huttenlocher PR, Hillman RE, Hsia YE. Pseudotumor cerebri in galactosemia. *J Pediatr* 1970; **76**: 902.
25. Belman AL, Moshe SL, Zimmerman RD. Computerised tomographic demonstration of cerebral edema in a child with galactosemia. *Pediatrics* 1986; **78**: 606.
26. Litman N, Kanter AI, Finberg L. Galactokinase deficiency presenting as pseudotumor cerebri. *J Pediatr* 1975; **86**: 410.
27. Fishler J, Donnell GN, Bergen WR, Koch R. Intellectual and personality development in children with galactosemia. *Pediatrics* 1972; **50**: 412.
28. Berger HM, Vlasveld L, Van Gelderen HH, Ruys JH. Low serum thyroxine concentrations in babies with galactosemia. *J Pediatr* 1983; **103**: 930.
29. Buist NRM, Nelson D, Tuerck JM. Dyspraxia in treated galactosemic patients. *Clin Res* 1983; **34**: 75.
30. Waggoner DD, Buist NRM, Donnell GN. Long-term prognosis in galactosaemia: results of a survey of 350 cases. *J Inher Metab Dis* 1990; **13**: 802.
31. Nelson MD Jr, Wolff JA, Cross CA et al. Galactosemia: evaluation with MR imaging. *Radiology* 1992; **184**: 255.
32. Lo W, Packman S, Nash S et al. Curious neurologic sequelae in galactosemia. *Pediatrics* 1984; **73**: 309.
33. Gitzelmann R, Hansen RG, Steinmann B. Biogenesis of galactose, a possible mechanism of self-intoxication in galactosemia. In: Hommes FA, Van den Berg CJ (eds). *Normal and Pathological Development of Energy Metabolism*. London: Academic Press, 1975: 25.
34. Brivet M, Migayron F, Roger J et al. Lens hexitols and cataract formation during lactation in a woman heterozygote for galactosaemia. *J Inher Metab Dis* 1989; **12**: 343.
35. Brivet M, Raymond JP, Konopka P et al. Effect of lactation in a mother with galactosemia. *J Pediatr* 1989; **115**: 280.
36. Tedesco TA, Wu JW, Bioches FS, Mellman WJ. The genetic defect in galactosemia. *N Engl J Med* 1975; **292**: 737.
37. Krooth R, Winberg AN. Studies on cell lines developed from the tissues of patients with galactosemia. *J Exp Med* 1961; **113**: 1155.
38. Dale GL, Popjak G. Purification of normal and inactive galactosemic galactose-1-phosphate uridytransferase from human red cells. *J Biol Chem* 1976; **251**: 1057.
39. Donnell GN, Bergren WR, Bretthauer MS, Hansen RG. The enzymatic expression of heterozygosity in families of children with galactosemia. *Pediatrics* 1960; **25**: 572.
40. Beutler E, Baluda MC. A simple spot screening test for galactosemia. *J Lab Clin Med* 1966; **68**: 137.
41. Nelson K, Hsia DYY. Screening for galactosemia and glucose-6-phosphate-dehydrogenase deficiency in newborn infants. *J Pediatr* 1967; **71**: 582.
42. Beutler E, Baluda MC, Sturgeon P, Day R. A new genetic abnormality resulting in galactose-1-phosphate uridytransferase deficiency. *Lancet* 1965; **1**: 353.
43. Mathai CK, Beutler E. Electrophoretic variation of galactose-1-phosphate uridytransferase. *Science* 1966; **154**: 1179.
44. Ng WG, Bergren WR, Donnell GN. A new variant of galactose-1-phosphate uridytransferase in man: the Los Angeles variant. *Ann Hum Genet* 1973; **37**: 1.
45. Segal S, Blair A, Roth H. The metabolism of galactose by patients with congenital galactosemia. *Am J Med* 1965; **38**: 62.
46. Matz D, Enzenauer J, Menne F. Über einen Fall von atypischer Galactosamie. *Humangenetik* 1975; **27**: 309.
47. Ng WG, Bergren WR, Field M, Donnell GN. An improved electrophoretic procedure for galactose-1-phosphate uridytransferase: demonstration of multiple activity bands with the Duarte variant. *Biochem Biophys Res Commun* 1969; **37**: 354.
48. Shin YS, Niedermeier HP, Endres W et al. Agarose gel isoelectrofocusing of UDP-galactose pyrophosphorylase and galactose-1-phosphate uridytransferase. Developmental aspect of UDP-galactose pyrophosphorylase. *Clin Chim Acta* 1987; **166**: 27.
49. Shin YS, Rieth WE, Schaub J. Prenatal diagnosis of galactosemia and properties of galactose-1-phosphate uridytransferase in erythrocytes of galactosemic variants as well as in human fetal and adult organs. *Clin Chim Acta* 1983; **128**: 271.
50. Levy HL, Sepe SJ, Walton DS et al. Galactose-1-phosphate uridytransferase deficiency due to Duarte/galactosemia combined variation: Clinical and biochemical studies. *J Pediatr* 1978; **92**: 390.
51. Kelly S. Significance of the Duarte/classical galactosemia genetic compounds. *J Pediatr* 1979; **94**: 937.
52. Schwarz HP, Zuppinger KA, Zimmerman A et al. Galactose intolerance in individuals with double heterozygosity for Duarte variant and galactosemia. *J Pediatr* 1982; **100**: 704.
53. Donnell GN, Bergeron WC, Ahi O, Golbus MS. Prenatal diagnosis of galactosemia. *Clin Chim Acta* 1977; **74**: 227.
54. Jakobs C, Warner TG, Sweetman L, Nyhan WL. Stable isotope dilution analysis of galactitol in amniotic fluid: an accurate approach to the prenatal diagnosis of galactosemia. *Pediatr Res* 1984; **18**: 714.

55. Levy HL. Screening for galactosemia. In: Burman D, Holton JB, Penneck CA (eds). *Inherited Disorders of Carbohydrate Metabolism*. Lancaster: MTP Press, 1980: 133.
56. Tedesco TA. Human galactose-1-phosphate uridyl transferase. *J Biol Chem* 1972; **247**: 6631.
57. Mohandas T, Sparkes RS, Sparkes MC, Schulkin JD. Assignment of the human gene for galactose-1-phosphate uridyltransferase to chromosome 9: studies with Chinese hamster-human somatic cell hybrids. *Proc Natl Acad Sci USA* 1977; **74**: 5628.
58. Mohandas T, Sparkes RS, Sparkes MC et al. Regional localization of human gene loci on chromosome 9: studies of somatic cell hybrids containing human translocations. *Am J Hum Genet* 1979; **31**: 586.
59. Sparkes RS, Sparkes MC, Funderburk SJ, Moedjono S. Expression of GALT in 9 p chromosome alterations: assignment of GALT locus to 9 cen→9p22. *Ann Hum Genet* 1980; **43**: 343.
60. Reichardt KV, Berg P. Cloning and characterization of a cDNA encoding human galactose-1-phosphate uridyl transferase. *Mol Biol Med* 1988; **5**: 107.
61. Elsas LJ, Langley S, Paulk EM et al. A molecular approach to galactosemia. *Eur J Pediatr* 1995; **154**(Suppl. 2): S21.
62. Elsas LJ, Fridovich-Keil JL, Leslie N. Galactosemia: a molecular approach to the enigma. *Int Pediatr* 1993; **8**: 101.
63. Flach JE, Reichardt JKV, Elsas LJ. Sequence of a cDNA encoding human galactose-1-phosphate uridyl transferase. *Mol Biol Med* 1990; **7**: 365.
64. Leslie ND, Immerman EB, Flach JE et al. The human galactose-1-phosphate uridyl transferase gene. *Genomics* 1992; **14**: 474.
65. Elsas LJ, Dembure PP, Langley S et al. A common mutation associated with the Duarte Galactosemia allele. *Am J Hum Genet* 1994; **54**: 1030.
66. Carney AE, Sanders RD, Garza KR et al. Origins, distribution and expression of the Duarte-2 (D2) allele of galactose-1-phosphate uridylyltransferase. *Hum Mol Genet* 2009; **18**: 1624.
67. Elsas LJ, Langley S, Steele E et al. Galactosemia: a strategy to identify new biochemical phenotypes and molecular genotypes. *Am J Hum Genet* 1995; **56**: 630.
68. Sakura N, Mizoguchi N, Ono H et al. Congenital porto-systemic shunt as a major cause of galactosemia. *Int Pediatr* 2001; **16**: 206.
69. Borden M. Screening for metabolic disease. In: Nyhan WL (ed.). *Abnormalities in Amino Acid Metabolism in Clinical Medicine*. Norwalk, CT: Appleton Century Crofts, 1984: 401.
70. Dhalqvist A. Test paper for galactose in urine. *Scand J Clin Lab Invest* 1968; **22**: 87.
71. Schwartz V, Golberg L, Komrower GM, Holzel A. Some disturbances of erythrocyte metabolism in galactosaemia. *Biochem J* 1956; **62**: 34.
72. Van Heyningen R. Galactose cataract: a review. *Exp Eye Res* 1967; **11**: 415.
73. Gitzelmann R. Hereditary galactokinase deficiency a newly recognized cause of juvenile cataracts. *Pediatr Res* 1967; **1**: 14.
74. Datiles F, Fukui H, Kuwabara T, Kinoshita JH. Galactose cataract prevention with sorbinil and aldose reductase inhibitor: a light microscopic study. *Invest Ophthalmol Vis Sci* 1982; **2**: 174.
75. Berry GT, Hunter JV, Wang Z et al. In vivo evidence of brain galactitol accumulation in an infant with galactosemia and encephalopathy. *J Pediatr* 2001; **138**: 260.
76. Kaufman FR, Xu YK, Ng WG, Donnell GN. Correlation of ovarian with galactose-1-phosphate uridyl transferase levels in galactosemia. *J Pediatr* 1988; **112**: 754.
77. Robertson A, Singh RH, Guerrero NV et al. Outcomes analysis of verbal dyspraxia in classic galactosemia. *Genet Int Med* 2000; **2**: 142.
78. Coman DJ, Murray DW, Byrne JC et al. Galactosemia, a single gene disorder with epigenetic consequences. *Pediatr Res* 2010; **67**: 286.
79. Donnell GN, Bergren WR. The galactosemias. In: Raine DW (ed.). *The Treatment of Inherited Metabolic Disease*. Lancaster: MTP Press, 1975: 91.
80. Koch R, Acosta P, Donnell GN, Lieberman E. Nutritional therapy of galactosemia. *Clin Pediatr (Phil)* 1965; **4**: 571.
81. Gitzelmann R. Alpha-D-galactose-1-phosphate determination as galactose after hydrolysis of phosphate. In: Bermeyer HU, Gawelin K (eds). *Methods of Enzymatic Analysis*. New York: Weinheim, Academic Press, 1974: 1291.
82. Doyle CM, Channon S, Orlowska D et al. The neuropsychological profile of galactosaemia. *J Inherit Metab Dis* 2010; **33**: 603.
83. Ficicioglu C, Thomas N, Yagera C et al. A pilot study of biochemical and neurodevelopmental assessment in children detected by newborn screening. *Mol Genet Met* 2008; **95**: 206.
84. Ficicioglu C, Hussa C, Gallagher PR et al. Monitoring of biochemical status in children with Duarte galactosemia: utility of galactose, galactitol, galactonate, and galactose 1-phosphate. *Clin Chem* 2010; **56**: 1177.

Glycogen storage diseases: introduction

The glycogen storage diseases are characterized by the deposition of glycogen in tissue cells. They are a heterogeneous group with different etiologies and different clinical manifestations. The classic form of glycogen storage disease was first described by von Gierke in 1929 [1]. The glycogen from this original patient was isolated by Schönheimer [2] and was found not to differ from normal glycogen in optical rotation or in its composition of glucose residues. The resistance of this material to glycogenolysis by the patient's liver *in vitro* and its prompt degradation by normal liver led Schönheimer to the conclusion that an enzyme essential to glycogenolysis was missing. This appears to have been the first demonstration of the concept proposed by Garrod [3] that inborn errors of metabolism result from genetically determined deficiencies of single enzymes. The demonstration by Cori and Cori [4] of the virtual absence of the activity of glucose-6-phosphatase in livers of patients with classic von Gierke disease established the deficiency of a single enzymatic step in carbohydrate metabolism as the basis of this disease.

Glycogen is a branched, polydisperse molecule that has been recognized since the time of Claude Bernard as the storage form for carbohydrates in animal tissues. This polysaccharide is composed entirely of units of α -D-glucose and the units are joined together in 1,4 and 1,6 linkages (Figure 58.1) to form molecules with molecular weights in the vicinity of 1 to 4 million. The branched, tree-like structure (Figure 58.2) was worked out through the elegant studies of Cori and Cori and their colleagues, using stepwise enzymatic degradation [5–7]. A free reducing group occurs at only one point. The straight chains of glucose residues are linked together by α -1,4 bonds; branching occurs through 1,6 linkages. In normal human glycogens, 6–8 percent of the glucose residues are joined to the rest of the molecule in α -1,6 linkage [8]. Glycogen contains seven tiers of branch points; the outer branches are terminated in nonreducing end groups [9].

The major pathway for the catabolism of glycogen is shown in Figure 59.1. The splitting of the 1,4 linkages in glycogen is catalyzed in the presence of inorganic phosphate by phosphorylase to yield glucose-1-phosphate [10]. The phosphorylase is activated by phosphorylation of serine, which is stimulated by glucagon and epinephrine [11].

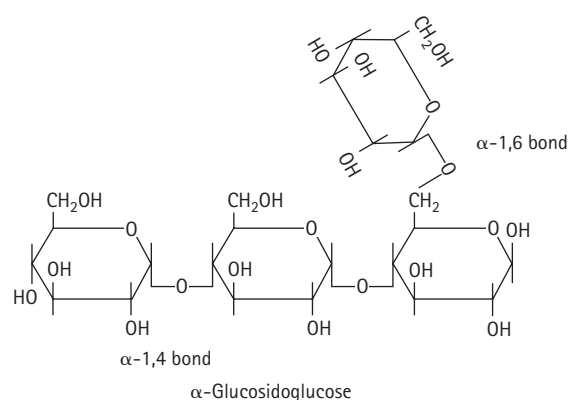


Figure 58.1 Portion of the glycogen molecule. The predominant structure is that of straight chains of glucose molecules in α -1,4 linkage. The branch points in the structure are created by α -1,6 linkages. Cleavage of the 1,4 bonds is catalyzed by phosphorylase and cleavage of the 1,6 bonds by amylo-1,6-glucosidase, the debranching enzyme.

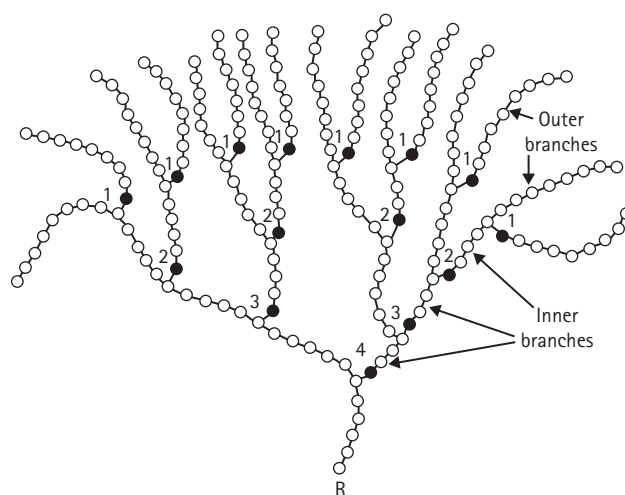


Figure 58.2 Structure of glycogen. The open circles represent glucose moieties in α -1,4 linkage and the black circles those in α -1,6 linkage at branch points. While four outer tiers of branch points are shown, glycogen has at least seven. R indicates the reducing end group. The outer branches terminate in nonreducing end groups. (Reproduced with permission from Cori [7].)

Phosphorylase kinase catalyzes the phosphorylation and activation of phosphorylase [12]. Removal of phosphate from the enzyme is catalyzed by protein phosphatase and inhibits phosphorylase activity.

The phosphorolysis of glycogen catalyzed by phosphorylase splits off glucose units until the 1,6 branch points are approached. These branches are degraded down to a limit dextrin in which three glucose residues are attached in 1,4 linkage to the 1,6-linked glucose. The transfer of this trisaccharide to the end of another glycogen chain is catalyzed by the transferase activity of the debranching enzyme. Then, the exposed glucose at the branch point is cleaved by the same enzyme protein in which amylo-1,6-glucosidase activity is at a different catalytic site [13]. The product of the reaction is free glucose. The combined activity of phosphorylase and the debranching enzyme accomplish the complete degradation of glycogen.

Glycogen is stored in liver and muscle, and these are the tissues predominantly affected in the classic glycogenosis. Pompe disease, or glycogenosis II, is an exception because it is a lysosomal storage disease. Its major effects are cardiac and there is no problem with this type of hepatic storage. The fact that enzymes, such as phosphorylase, have different genetically determined enzymes in liver and muscle leads to different diseases that have hepatic or myopathic clinical manifestations. Hepatic metabolism of glycogen is critical for glucose homeostasis; hepatic glycogenoses present classically with hypoglycemia. Muscle glycogen is used to make adenosine triphosphate (ATP) for contraction; glycogenoses of muscle present with cramps, weakness, stiffness, or rhabdomyolysis.

Eight numbered types of glycogenosis result from specific defects in enzymes of glycogen catabolism (Table 58.1). They were given numbers in the chronological order of their description. The numbers seem less useful now that molecular defects have been identified, leading to multiple different forms of type I, as well as the disappearance of type VIII, which was once used for phosphorylase kinase deficiency. Nevertheless, these numbers are in such common use, they will be continued.

1. The classic form of glycogen storage disease originally described by von Gierke [1] is caused by a deficiency of glucose-6-phosphatase (Chapter 60).
2. Type II, or Pompe disease, (Chapter 61) is a lysosomal storage disease which usually causes death in infancy from cardiomyopathy.
3. Glycogenosis type III (Chapter 62) is the result of defective activity of the debrancher enzyme. It causes massive hepatomegaly in infancy and progressive myopathy in adults.
4. Type IV, or Andersen disease, is a very different type of disorder in which defective activity of the debranching enzyme produces abnormal glycogen that appears to act as a foreign body and causes hepatic cirrhosis.
5. Type V, or McArdle disease, results from defective activity of phosphorylase in muscle. Symptoms are those of muscle cramps that limit exercise tolerance and myoglobinuria.
6. Hepatic phosphorylase deficiency, type VI [14], leads to a mild hepatic glycogen storage disease. Hepatomegaly may be the only clinical manifestation.

Table 58.1 Glycogen storage diseases

Enzyme deficiency	Glycogen structure	Organs	First description	Synonyms	Type
Glucose-6-phosphatase	Normal	Liver, kidney	von Gierke	Hepatorenal glycogenosis	Ia
Glucose-6-phosphate transport protein	Normal	Liver, neutrophils	Narasawa, Lange	Translocase T ₁ deficiency	Ib,c
Stabilizing protein	Normal	Liver	Burchell	Regulatory protein deficiency	Iasp
Microsomal glucose transporter	Normal	Liver	Leonard	GLUT7 deficiency	Id
α -1,4 Glucosidase	Normal	Cardiac	Pompe	Cardiac glycogenosis, generalized glycogenosis	II
Amylo-1,6-glucosidase (debrancher)	Abnormal: very short outer branches	Liver, muscle	Forbes	Limit dextrinosis	III
Amylo-(1,4 \rightarrow 6) trans-glucosidase (brancher)	Abnormal: long, straight chains	Liver, muscle	Andersen	Amylopectinosis	IV
Muscle phosphorylase	Normal	Muscle	McArdle	Myophosphorylase deficiency	V
Liver phosphorylase	Normal	Liver	Hers	Hepatophosphorylase deficiency	VI
Muscle phosphofructokinase	Normal	Muscle, erythrocytes	Tarui	–	VII
Phosphorylase kinase, 4 subunits ^a	Normal	Liver	Hug, Huijing	–	IX
GLUT2	Normal	Liver	Fanconi, Bickel	Glucose transporter	

^aAutosomal recessive and X-linked phosphorylase kinase deficiency diseases have been defined.

Table 58.2 Glycogen synthase deficiency

Fasting	Fed	Glucose tolerance test
Hypoglycemia, Increased acetoacetate 3-hydroxybutyrate Alanine and lactate low No response to glucagon	Normoglycemia, hyperglycemia Increased lactate after glucagon	Hyperglycemia, Hyperlactic acidemia, free fatty acids, acetoacetate 3-Hydroxybutyrate normal

7. Type VII glycogenosis, or Tarui disease, is clinically identical to McArdle disease, but the enzymatic defect is in the phosphofructokinase of muscle.
8. Phosphorylase kinase deficiency is now known as type IX, but this enzyme is composed of four subunits coded by different genes, and hence the inheritance of its five different clinical subtypes are variously autosomal recessive and X-linked recessive [15]. The most common of these affecting some three-quarters of type IX patients is the X-linked recessive defect in the α -subunit. The γ -subunit controls the catalytic center; the α -, β -, and δ subunits are regulatory. All but α are autosomal recessive. Patients with type IX disease often have elevations in transaminases. Those with defective activity in the A1 subunit leave detectable activity of the phosphorylase kinase in liver, erythrocytes, and leukocytes. On the other hand, those with mutations in the A2 subunit have normal kinase activity in erythrocytes and leukocytes, and sometimes even in liver.

Patients with type IX disease and with phosphorylase deficiency often present with isolated hepatomegaly and are often first referred to the metabolic service after a liver biopsy has identified large amounts of glycogen. Most do not require treatment and as adults have normal height and modest enlargement of the liver.

The Fanconi-Bickel syndrome combines a hepatic glycogen storage disease with a Fanconi syndrome pattern of renal tubular dysfunction [16]. Some have called it 'type XI'. Patients have failure to thrive and intolerance to galactose. These patients also resemble type I in that they have hyperlipidemia and hyperuricemia. The molecular defect is the liver type facilitated glucose/galactose transporter GLUT2.

Glycogen synthase deficiency (Table 58.2) is sometimes referred to as type 0 or GSDO, but this is a misnomer as there is no storage of glycogen. Patients have fasting hypoglycemia and ketosis; postprandially, they have elevation of lactic acid. They are often thought to have ketotic hypoglycemia. Glucose tolerance tests lead to substantial lactic acidemia. The enzyme activity can be tested only in liver. Current diagnosis is often made by a search for mutation.

REFERENCES

1. Von Gierke E. Hepato-nephromegaly glykogenica. *Beitr Path Anat* 1929; **82**: 497.
2. Schönheimer R. Über eine eigenartige Störung des Kohlenhydrat-Stoffwechsels. *Zeitschr Physiol Chem* 1929; **182**: 148.
3. Garrod AE. *Inborn Errors of Metabolism*. London: Oxford University Press, 1933.
4. Cori GT, Cori CF. Glucose-6-phosphatase of liver in glycogen storage disease. *J Biol Chem* 1952; **199**: 661.
5. Illingworth B, Cori GT. Structure of glycogens and amylopectins. III. Normal and abnormal human glycogens. *J Biol Chem* 1952; **199**: 653.
6. Larner J, Illingworth B, Cori GT, Cori CF. Structure of glycogens and amylopectins. II. Analysis by stepwise enzyme degradation. *J Biol Chem* 1952; **199**: 641.
7. Cori GT. Biochemical aspects of glycogen deposition disease. *Mod Prob Paediatr* 1957; **3**: 344.
8. Cori CF. The enzymatic synthesis and molecular configuration of glycogen. In: Najjar VA (ed.). *A Symposium on the Clinical and Biochemical Aspects of Carbohydrate Utilization in Health and Disease*. Baltimore, MD: Johns Hopkins Press, 1952.
9. Cori CF. Glycogen structure and enzyme deficiencies in glycogen storage disease. *Harvey Lectures* 1952–1953; **48**: 145.
10. Newgard CB, Littman DR, Van Genderen C *et al*. Human brain glycogen phosphorylase. Cloning sequence analysis chromosomal mapping tissue expression and comparison with the human liver and muscle isozymes. *J Biol Chem* 1988; **263**: 3850.
11. Cohen P, Hardie GG. The actions of cyclic AMP on biosynthetic processes are mediated indirectly by cyclic AMP dependent protein kinase. *Biochim Biophys Acta* 1991; **1094**: 292.
12. Francke U, Barras BT, Zander NF, Kilimann MW. Assignment of human genes for phosphorylase kinase subunits a (PHKA) to Xq12–q13 and b (PHBK) to 16q12–q13. *Am J Hum Genet* 1989; **45**: 276.
13. Liu W, Madsen NB, Braun C, Withers SG. Reassessment of the catalytic mechanism of glycogen branching enzyme. *Biochemistry* 1991; **30**: 1419.
14. Lerardi-Curto L. Glycogen storage disease type VI. *Eur J Pediatr* 1998; **157**: 919.
15. Van Den Berg IET, Berger R. Phosphorylase b kinase deficiency in man: a review. *J Inher Metab Dis* 1990; **13**: 442.
16. Santer R, Schneppenheim R, Suter D *et al*. Fanconi-Bickel syndrome – the original patient and his natural history, historical steps leading to the primary defect, and a review of the literature. *Eur J Pediatr* 1998; **157**: 783.

Glycogenosis type I – Von Gierke disease

Introduction	428	Treatment	433
Clinical abnormalities	429	References	434
Genetics and pathogenesis	432		

MAJOR PHENOTYPIC EXPRESSION

Hypoglycemia; massive hepatomegaly from storage of glycogen in the liver; short stature; prolonged bleeding time; ketosis, hyperlipidemia, lactic acidemia, and hyperuricemia; late complications of gout, hepatic adenomas, renal disease, and osteoporosis; impaired glycemic response to glucagon; and deficient activity of hepatic glucose-6-phosphatase.

INTRODUCTION

The classic form of glycogen storage disease originally described by von Gierke [1] is caused by a deficiency of glucose-6-phosphatase (Figure 59.1). It became apparent that there were subtypes of glycogenosis I and a considerably expanded glucose-6-phosphatase system when patients were studied who appeared to have von Gierke disease in which glucose-6-phosphatase activity in frozen liver was normal. The term glycogenosis type Ib was derived to distinguish these patients from those (Ia) in whom the activity of the enzyme is deficient [2, 3]. In 1978, Narisawa and colleagues [3] found defective glucose-6-phosphatase activity in fresh liver and restored activity by adding detergents; they suggested that the defect was in

glucose-6-phosphate transport. The translocase defect was reported in 1980 by Lange and colleagues [4]. The gene has been mapped to 11q23-24.2 [5]. Type Ic was recognized [5] on the basis of normal activity of glucose-6-phosphatase in detergent-disrupted microsomes, while activity in intact microsomes is defective for both glucose-6-phosphate and carbamylphosphate substrates, but molecular studies have indicated that both Ib and Ic are caused by mutations in translocase. Mutations in the gene have been found in the patients with type Ib or Ic disease [6]. Type Id with defective microsomal transport of glucose has not yet been observed clinically. A variant of type Ia is a result of the deficiency of the regulatory protein, designated Iasp, for stabilizing protein which has so far been reported in a single patient [7]; it is impossible to distinguish this from deficiency of

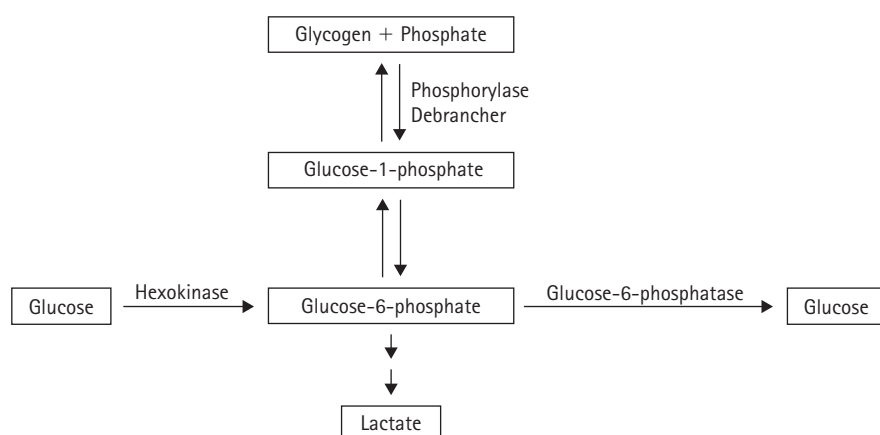


Figure 59.1 Glucose-6-phosphatase, the site of the defect in von Gierke disease.

the catalytic subunit clinically, and difficult biochemically unless the entire stabilizing protein is missing.

The enzyme, glucose-6-phosphatase [8] is expressed in liver, but also in kidney, pancreatic islets, and intestinal mucosa; and glycogen accumulates in all three organs. Clinical manifestations appear largely, if not entirely, consequences of the metabolic effects of the enzyme defect. Late effects, hepatic adenomas, and renal disease are of uncertain pathogenesis. The enzyme is situated in the endoplasmic reticulum, and this sets the stage for transport defects.

The gene for glucose-6-phosphatase has been cloned [9] and so has that of the translocase [10]. Mutations have been identified in the phosphatase [9], some of which are specific for certain ethnic groups, such as R83C and Q347X in Caucasians and G727T in Japanese [11].

CLINICAL ABNORMALITIES

In classic type Ia glycogen storage disease, symptoms usually occur in the first months of life, and the disease may be recognized at birth. There may be neonatal hypoglycemia. Hepatomegaly is often present at birth [12] and progresses to huge enlargement of the liver without splenomegaly (Figures 59.2, 59.3, 59.4, 59.5, 59.6, and 59.7). The kidneys are enlarged too and may be visualized on roentgenography or may even be palpable. It is common in this condition for the liver to be palpable at the iliac crest in infancy and early childhood. The abdomen is protuberant, the posture lordotic (Figure 59.4), and the gait broad-based and rolling or swinging, all apparent consequences of the

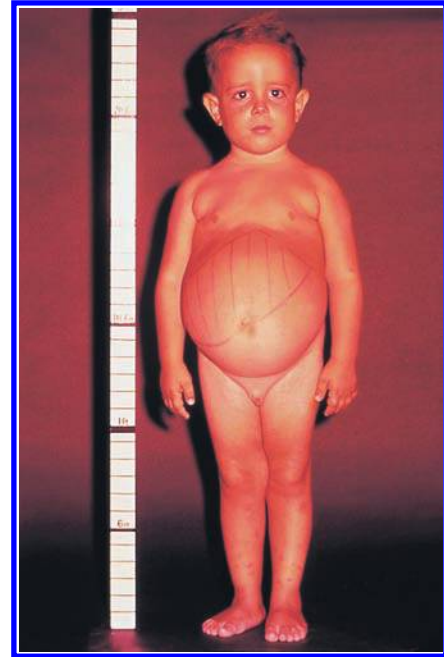


Figure 59.3 DA: A five-year-old boy with glucose-6-phosphate deficiency. He had massive hepatomegaly. There was some adiposity about the face.

hepatomegaly. With time and growth, the abdomen tends to become less prominent.

Linear growth is usually impaired (Figure 59.6). The shortness of stature is symmetrical in that there is proportionate reduction in the length of the trunk and extremities [13]. Adiposity, particularly about the cheeks,



Figure 59.2 OH: An infant with glycogen storage disease type I. The face somewhat chubby and the liver decidedly enlarged.

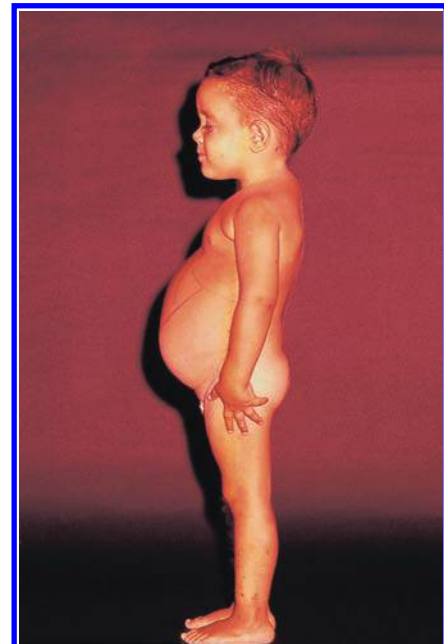


Figure 59.4 The abdominal enlargement is more impressive in the lateral view.



Figure 59.5 A six-month-old infant with glycogen storage disease (GSD) type I. He had the typical chubby cheeks, hepatic enlargement, and abdominal distention. He had hypoglycemia and acidosis. Diagnosis was made following liver biopsy. Depigmented spots were cautery marks, resulting from a common practice in the Middle East.

is the cause of the doll-like or cherubic appearance (Figures 59.3, 59.5, and 59.7). Musculature tends to be flabby and poorly developed [14] and the legs appear thin.

Hypoglycemic symptoms tend to appear after three to four months, when the infant begins to sleep through the night. Nevertheless, symptomatic hypoglycemia may present in early infancy [15] or even in the neonatal period [16]. Manifestations of low blood sugar may include irritability, pallor, insomnia, feeding difficulties, and seizures. Episodes of hypoglycemia and metabolic acidosis in the neonatal period may be refractory to treatment [15], or so easily controlled that the diagnosis is not entertained until much later. Many infants present with vomiting or convulsions in the morning [17]. These symptoms are relieved by feeding or by the administration of glucose, and frequent feedings prevent their recurrence. With increase in the activity of the child at about one year of age, the frequency of hypoglycemic symptoms tends to increase. As in the case of any hypoglycemic syndrome, severe convulsions and permanent brain injury may be seen in patients with this disease.

In a series of 19 patients studied specifically for evidence of brain damage [18], abnormalities of the EEG were found in four, only two of whom had seizures; some had abnormal evoked potentials, and all of these abnormalities correlated inversely with compliance with treatment. Abnormalities in magnetic resonance imaging (MRI) of the brain in



Figure 59.6 MS: A 60-year-old woman with glycogenosis type I. She was short. The scar represented recent surgical removal of a hepatoma. Abdominal girth was markedly reduced by the procedure.



Figure 59.7 MS: Facial appearance was still rounded. She appeared considerably younger than her chronological age.

seven correlated with neonatal hypoglycemia. None had impaired mental development. Children with this disease have an unusual degree of tolerance to hypoglycemia, often appearing quite well at levels of blood sugar at which convulsions would ordinarily be expected [12], and the incidence of impaired mental development is not high. Possibly this is a function of chronic hypoglycemia, where the rates of change of blood glucose are not very fast, but also parents and children become quite aware of the limits of tolerance for fasting. It could be relevant to utilization of lactic acid or ketones by the brain. Some patients are asymptomatic, discovered only by the presence of hepatomegaly on routine physical examination [15, 19], and one of our patients came to attention at surgery for an adenoma, with evidence of glycogen storage in the adjacent liver.

Bleeding may be a major clinical manifestation in patients with this disease. It may take the form of frequent nosebleeds in which there is considerable loss of blood. Abnormal hemostasis and persistent oozing may complicate surgery. These problems are thought to represent defective platelet function. Bleeding time and platelet adhesion are abnormal, and there is defective collagen and epinephrine-induced aggregation of platelets [20]. Many small superficial vessels may be visible under the thin-looking skin.

Intermittent episodes of diarrhea have been reported [14, 15, 21]. This has been attributed to malabsorption of glucose [22], but in other studies no evidence was found of malabsorption of monosaccharides, disaccharides, or fat [14, 15, 21]. Intestinal biopsy revealed no signs of inflammation and fecal α -1 antitrypsin was not increased [23]. One patient died from hemorrhagic pancreatitis [24].

Cutaneous xanthomas may develop over the buttocks, hips, elbows, and knees [25, 26]. Their occurrence is related to the elevated levels of triglycerides in the blood. Another consequence of hyperglycemia is the appearance of characteristic discrete, flat, yellowish retinal lesions [27] in the paramacular area; they do not adversely affect vision [18]. Patients with untreated hyperuricemia develop tophaceous gout [26, 28]. Most patients have osteoporosis and repeated spontaneous fractures have been seen in some patients [25].

In addition to the hypoglycemia, a variety of abnormalities can be detected in the clinical chemistry laboratory. Lactic acidemia is a regular feature of the disease [29], and occasionally the level of pyruvate is increased. Marked hyperlipidemia and hypercholesterolemia are also features of the disease [12, 30]. Concentrations of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) are high, and the apolipoproteins apoB, C, and E are high, while apoA and D are normal or low [31]. The hyperlipidemia leads not only to the formation of xanthomas, but also to large lipid-laden reticuloendothelial cells in the bone marrow. The plasma may be milky. It is important to recognize that the concentration of water in serum or plasma is markedly reduced in the presence of hyperlipidemia. In as much as electrolytes and other

substances are distributed only in the water phase, extremely low values are recorded for the serum sodium and other constituents. A correction must be made for the increased quantity of serum solids and decreased serum water in order to avoid a mistaken diagnosis of hyponatremia.

Ketosis and ketonuria occur promptly with minimal degrees of fasting [30]. In fact, glucose-6-phosphatase deficiency is one of the few conditions in which ketones may be observed in the urine in the neonatal period. This and the lactic acidosis concomitantly may lead to metabolic acidosis. Despite the fact that ketosis has long been considered a characteristic of the disease, patients with this disease have been reported [32, 33] to be resistant to ketosis or to have decreased ketogenesis. Dicarboxylic aciduria has been observed [34] and this would be consistent with a suppression of the β -oxidation of fatty acids leading to ω -oxidation. Hyperuricemia is a regular concomitant of the disease [28]. This has been attributed to competition by lactic acid for renal tubular secretion and decreased clearance of uric acid has been observed. However, not all patients have reduced clearance of uric acid [35], and studies of uric acid production have provided evidence of increased purine synthesis in this disease [28, 36, 37]. A renal Fanconi syndrome of glycosuria, aminoaciduria, and phosphaturia has been reported in a number of patients with von Gierke disease [38, 39], but at least one of these patients [38] is now known to have Fanconi-Bickel disease. An unusual manifestation is elevation of biotinidase activity [40]. In two patients previously not diagnosed, biotinidase levels were 26 and 15 mmol/min/mL (normal mean, 7); in the second, it was this that led to the diagnosis.

The administration of epinephrine or glucagon fails to provoke the normal hyperglycemic response [41]. There may be some elevation of blood glucose after these agents, even in the virtual absence of glucose-6-phosphatase, for 6–8 percent of the glucose residues of glycogen are released as free-glucose as the product of the debranching enzyme. In response to glucagon or epinephrine, there is a marked increase in the concentration of lactic acid in the blood. In the absence of glucose-6-phosphatase, there is also a failure of the usual rise in blood glucose following the administration of galactose or fructose [15]. Similarly, following the intravenous administration of glycerol, elevation of blood glucose was less than normal and levels of lactic acid rose [42]. These abnormal responses to epinephrine, glucagon, fructose, glycerol, or galactose have, in the past, been used to diagnose the condition. Today, the glucagon test is employed for preliminary diagnosis and decision about candidacy for liver biopsy, and the definitive finding is the assay of the enzyme and the demonstration of the accumulation of glycogen in the cells of the liver (Figure 59.8). The existence of some common mutations means it is now possible to make a definitive diagnosis by mutational analysis and avoid biopsy of the liver.

Pathologic examination [1, 43] (Figure 59.8) reveals that the hepatocytes and renal tubular cells are swollen and clear as stained with hematoxylin and eosin; staining with

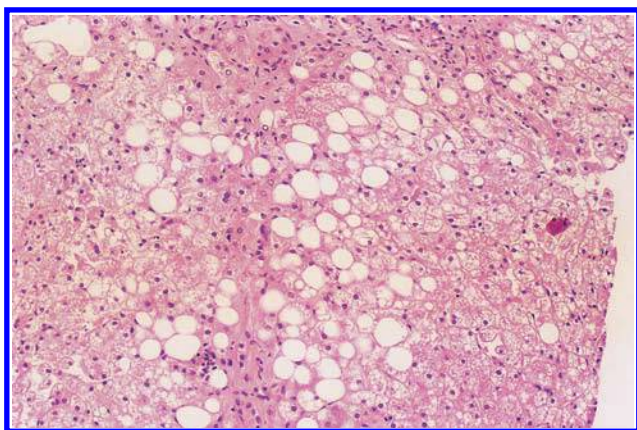


Figure 59.8 Biopsied liver of AK, a boy with glycogenosis type I. He clearly had storage of glycogen in distended hepatocytes, but there was also so much lipid that the initial pathologic diagnosis of Wolman disease was only discarded after the acid lipase was found to be normal, and he was referred to the metabolic service.

Best's carmine reveals the stored material to be glycogen. There may also be extensive lipid storage in the liver. In fact, the lipid may be so prominent that the referring pathologic diagnosis may be lipid storage disease, such as Wolman disease (Chapter 97). In the neonatal period, the histology of the liver can be so normal that one is led away from the diagnosis [16].

Chemical analysis establishes that the material stored in the liver and kidneys is glycogen. Biopsy of the liver with demonstration of a glycogen content over 12 percent of the wet weight of the liver has been set out [30] as a criterion for the diagnosis of this disease. Schoenheimer [44] did not find quite that much glycogen, and most investigators would find values over 4 percent acceptable, but again, today the gold standard for diagnosis in biopsied liver is assay of the enzyme. The structure of the glycogen stored is normal [45].

A number of late complications have been observed, particularly as these patients reach adulthood. Hyperuricemia is present in infancy, but symptomatic gout occurs after adolescence. Pancreatitis [24, 46] appears to be a consequence of the hypertriglyceridemia. Adult stature is low and puberty is often delayed. Fertility is not affected, and both males and females have had children [47].

An appreciable problem in management has been the regular development of hepatic adenomas by the second or third decade [48]. These nodules are multiple, and they grow, sometimes to sizable tumors. They are usually benign, but transformations to malignant hepatocellular carcinomas have been recorded and may be fatal [48, 49]. Another complication is bleeding into the adenoma [50]. Patients should therefore be investigated at intervals for these nodules, and once present their size and character should be followed. Scintigraphic scans have been

recommended for this purpose [51], but ultrasonography and other forms of imaging have been useful in our hands.

A variety of renal complications has been observed. In addition to the renal Fanconi syndrome, patients have had distal renal tubular disease [52], amyloidosis [53], hypercalciuria, nephrocalcinosis, and calculi. Decrease in urinary citrate was found in 15 patients [54], reversing the normal increase with age, along with hypercalciuria. The authors suggested that treatment with citrate might be useful in preventing nephrocalcinosis and calculi. Glomerular hyperfiltration, increased renal plasma flow, and microalbuminuria [55] are followed over time by proteinuria, focal segmental glomerulosclerosis, and interstitial fibrosis [23, 56, 57]; followed in some patients by renal failure, leading to dialysis and transplantation.

Glycogenosis type Ib/c

Patients with glycogen storage disease type Ib [4] have the same type I clinical phenotype, but in addition they have neutropenia and impaired neutrophil function (see Table 58.1 in Chapter 58) [58]. As a consequence, they have recurrent bacterial infections, inflammatory bowel disease, and ulceration of oral and intestinal mucosa [59–63]. Fecal α -1 antitrypsin is increased, biopsy of the colon reveals inflammation [23]. Among 288 patients with type I glycogenosis, 57 had type Ib [62]. Neutropenia ($<1 \times 10^9/L$) was found in 54. It is often documented in the first year of life, but may be noted first between six and nine years. It may be persistent, but more commonly it is intermittent. Among 18 patients with neutropenia in whom neutrophil function was studied, it was abnormal in all. Apoptotic neutrophils were documented by increased activity of caspase, condensed nuclei, and perinuclear clusters of mitochondria in this disease, but not in other neutropenic disorders, such as Shwachman Diamond syndrome [64]. Perioral infections occurred in 37 patients, perianal infections in 27, and protracted diarrhea in 23. Inflammatory bowel disease was documented by colonoscopy or roentgenographic examination. Inflammatory bowel disease was not observed in the absence of neutropenia. Two Japanese patients were reported with no evidence of neutropenia and no recurrent bacterial infections [63]; otherwise, symptoms were typical of type Ib glycogenosis and there were mutations on both alleles of the gene.

GENETICS AND PATHOGENESIS

The disorder is inherited as an autosomal recessive trait. It has frequently been observed in siblings and as a concomitant of parental consanguinity [12, 25, 46, 65]. The distribution between the sexes is about equal. Ethnic differences in the severity of disease have been observed.

For example, patients from Syria and Lebanon tend to have serious disease, while those of Saudi Arabia have had quite mild disease. Reduced activity of intestinal glucose-6-phosphatase has been found in parents of patients [66]. Prenatal diagnosis has been made by biopsy of the fetal liver and enzyme assay [67].

The molecular defect in glycogenosis I is absence of activity of the catalytic subunit of the glucose-6-phosphatase enzyme complex [2]. The enzyme is expressed normally in liver, kidney, and in the β cells of pancreatic islets. The diagnosis has generally been made by assay of biopsied liver. Absence or near absence of the enzyme has been required for the diagnosis [68, 69], because many enzyme activities, including this one, may be reduced in liver disease or in other storage diseases. The diagnosis can be made by needle biopsy, but sufficient complexity has been recognized that open biopsy and an adequate sample of tissue are preferred. Direct vision also protects against bleeding. Samples should also be fixed for light and electron microscopy. Care should be taken to avoid destruction of hepatic cellular membrane elements, and precautions for the handling of specimens prior to assay have been set out [5].

The active site of the enzyme is within the lumen of the endoplasmic reticulum [70]. Normal activity of the enzyme requires the activity of six different proteins or subunits in the enzyme complex [71, 72]. The discovery of these components and the elucidation of the function of the complex were the results of the study of patients with glycogen storage disease. The classic enzyme, or the catalytic subunit whose deficiency causes type Ia, is a 36.5-kDa protein [72] that catalyzes the hydrolysis of a number of phosphate compounds, including carbamylphosphate and pyrophosphate, as well as glucose-6-phosphate [73, 74]. A microsomal regulatory protein has been isolated as a 21-kDa stabilizing protein because it stabilizes the activity of the catalytic protein during purification [75]. It binds calcium and is essential for normal activity. Its deficiency leads to glycogenosis type Iasp [7].

The microsomal glucose-6-phosphate transport protein (T1; translocase) was recognized through the study of glycogenosis type Ib. T1 catalyzes the transport of glucose-6-phosphate into the lumens of hepatic microsomes [76, 77]. In its absence, the liver is unable to release glucose from glucose-6-phosphate. The glucose-6-phosphatase catalytic protein is normal and can be assayed if membranous elements of the liver cell are disrupted by freezing or treatment with detergents, but *in situ* the system is nonfunctional [3, 78, 79]. The defect is also demonstrable in leukocytes, which have impaired uptake of glucose [80] in type Ib, and this may provide a way to test for the disorder. Activity against pyrophosphate and carbamylphosphate is not impaired.

The glucose-6-phosphatase system also depends on the transport of glucose. A number of glucose transport proteins has been identified and they have been designated GLUT 1–6. Deficiency of GLUT 2 causes the

syndrome of hepatic glycogenosis in the Fanconi-Bickel syndrome [81].

In glycogenosis Ia, immunochemical assay has indicated an absence of glucose-6-phosphatase catalytic enzyme protein in some patients in whom there is little or no activity, but most have had a normal amount of a protein of normal size [82]. Among patients with partial deficiencies, some have reduced levels of immunoprotein and others normal amounts [14, 82, 83]. In some, the K_m is elevated.

The gene for human hepatic glucose-6-phosphatase on chromosome 17q21 has been cloned, and a number of mutations has been identified [84]. These include an arginine-to-cysteine change at amino acid 83 (p.R83C) and p.Q347X a change from a glutamine to a stop codon, which are common in Caucasians. p.R83C is also common in Hispanic and Jewish patients. The most prevalent mutation in Japanese was p.G727T [85]; the next most prevalent was p.R170X.

The cDNA for the translocase deficient in Ib has been cloned [10] and localized to chromosome 11q23 [86]. Common gene mutations in Caucasian patients are G339C and 1211delCT [87]. W118R is common in Japanese [88]. Of the two Japanese patients with type Ib without neutropenia, one had R415X, which had previously been encountered in patients with neutropenia, on one allele and G339D on the other. The other patient was homozygous for G794A, which led to a splicing error, deleting exon 3. Another patient with neutropenia, with abnormal neutrophil function and recurrent infections typical of Ib, was found to have no mutations in the translocase, but to be homozygous for G188R in the glucose-6-phosphatase gene; so she had type Ia [89]. In a study of adenomas in glycogenosis Ia, alterations in chromosome 6, such as a gain of 6p and a loss of 6q were prominent [90]. Tumors with changes in chromosome 6 tended to be larger than those without.

TREATMENT

Since patients with glycogenosis type Ia are at risk of death or hypoglycemic damage to the brain in early infancy, prompt diagnosis, the avoidance of fasting and the provision of free-glucose are important in getting the patient through this critical period. Infections are particularly dangerous, and the patient may require admission to hospital and treatment with parenteral fluids containing glucose and electrolytes. There is a distinct tendency for improvement with age, even by the age of four or five years [17, 30]. By the time of puberty, considerable amelioration has often been observed [12]; the enlarged liver takes up considerably less of the abdomen and hypoglycemic symptoms are much less prominent. However, little improvement in long-term prognosis as a result of treatment occurred until recently. Portacaval shunting has essentially been abandoned in the treatment

of this disease, but it was noted that the parenteral alimentation attendant on the procedure led to reduction in hepatic size and reversal of metabolic abnormalities, the growth failure and the bleeding diathesis [91, 92]. These observations focused attention on approaches to the more regular provision of glucose to meet tissue needs. The approaches that have been successful are continuous nocturnal nasogastric or gastrostomy feeding [92–95] and oral uncooked cornstarch [95, 96].

With either regimen, frequent high carbohydrate meals in which 65–70 percent of the calories are carbohydrate are employed during the day. Dietary intake of fructose and galactose is restricted in some centers and not in others.

Uncooked cornstarch provides glucose in a slow-release fashion. Use in an infant is recommended at a dose of 1.6 g/kg every 4 hours. In older children, the requirement is 1.75–2.5 g/kg every 6 hours, prepared in a 1:2 weight:volume mixture with water or diet drinks [97–100]. Argo (PC International) is prepared by suspension at room temperature. The optimal amount for each patient is determined individually; satisfactory results have been confirmed with 1 g/kg every 6 hours. Older patients may not require a feeding in the middle of the night if larger quantities (2–4 g/kg) can be taken at bedtime. This regimen has been shown to maintain euglycemia and to reverse clinical and biochemical disturbances in most patients [97, 98]. The age at which cornstarch may be introduced is controversial. Some start at six months and some at 12 months, employing maltodextrin prior to that. We have tended to begin with Polycose®.

Nocturnal nasogastric feeding has also been introduced in infancy at diagnosis. Glucose, Polycose, and elemental formulations have been employed, each providing 8–10 mg/kg per minute in an infant and 5–7 mg/kg per minute in an older child. Clinical and biochemical abnormalities can be reversed and hypoglycemia avoided. Liver size regresses and the bleeding tendency is reversed [101]. There is a tendency to the development of hypoglycemia in the morning after the nocturnal feeding is stopped; so that the first meal should be within 15–30 minutes of discontinuing the nocturnal feeding. Hypoglycemia and death have been reported following malfunction of the pump or dislodging the tube [101]. Some patients have required a combination of cornstarch and nocturnal nasogastric feedings. Patients with glycogenosis type Iasp and Ib should also be managed with these regimens. In type Ib, granulocyte and granulocyte–macrophage colony-stimulating factors have been employed to combat the neutropenia and treat the inflammatory disease [102].

Both regimens have been employed long enough to have provided [103] encouraging long-term effects on the course of the disease [97, 98, 103, 104]. Growth has been rewarding, and it is clear that normal adult height may be reached. Some children have remained short [103]. Surprisingly, treatment has been reported to be associated with an absence of development of hepatic adenomas [99]

and regression of adenomas has been observed. Proximal renal tubular function has improved [98]. Whether treatment will prevent glomerular dysfunction and renal failure is not clear.

Guidelines for the management of glycogenosis type I, are based on consensus of the European study [105]. Overall recommendations are for 60–65 percent of total calories from carbohydrates and 10–15 percent from fat.

Allopurinol is used to lower the concentration of urate to normal levels. A starting dose of 10 mg/kg was recommended. In patients requiring surgery, the bleeding time should be determined. If it is prolonged, it may be improved by 24–48 hours of intravenous glucose or L-deamino-8-D-arginine vasopressin (DDAVP) [106]. Angiotensin converting enzyme inhibitors have been recommended [105] for hypertension. Hypertriglyceridemia has been treated with nicotinic acid or fibrates [105].

Renal transplantation performed because of renal failure did not improve glucose homeostasis in this disease [107]. Transplantation of the liver provides a definitive cure of the disease [108, 109]; however, the magnitude of the procedure has tended to make its use reserved for a small number of patients with refractory disease or, of course, hepatic malignancy.

REFERENCES

1. Von Gierke E. Hepato-nephromegalie glykogenica. *Beitr Path Anat* 1929; **82**: 497.
2. Cori GT, Cori CF. Glucose-6-phosphatase of the liver in glycogen storage disease. *J Biol Chem* 1952; **199**: 661.
3. Narisawa K, Igarashi Y, Otomo H, Tada K. A new variant of glycogen storage disease type 1 probably due to a defect in the glucose-6-phosphate transport system. *Biochem Biophys Res Commun* 1978; **83**: 1360.
4. Lange AJ, Arion WJ, Beaudet AL. Type 1b glycogen storage disease is caused by a defect in the glucose-6-phosphate translocase of the microsomal glucose-6-phosphatase system. *J Biol Chem* 1980; **255**: 8381.
5. Fenske CD, Jeffery S, Weber JL *et al*. Localisation of the gene for glycogen storage disease type 1c by homozygosity mapping to 11q. *J Med Genet* 1998; **35**: 269.
6. Visser G, Herwig J, Rake JP *et al*. Neutropenia and neutrophil dysfunction in glycogen storage disease type 1c. *J Inher Metab Dis* 1998; **21**: 227.
7. Burchell A, Waddell ID. Diagnosis of a novel glycogen storage disease: type 1asp. *J Inher Metab Dis* 1990; **13**: 247.
8. Nordlie R, Sukalski K. Multifunctional glucose-6-phosphatase: a critical review. In: An M (ed.). *The Enzymes of Biological Membranes*. New York: Plenum Press, 1985: 349.
9. Lei K, Shelly L, Pan C *et al*. Mutations in the glucose 6-phosphatase gene that cause glycogen storage disease type Ia. *Science* 1993; **262**: 580.
10. Gerin I, Veiga-da-Cunha M, Achouri Y *et al*. Sequence of a putative glucose 6-phosphate translocase mutated in

- glycogen storage disease type Ib. *Fed Eur Biochem Soc* 1997; **419**: 235.
11. Chou J, Mansfield B. Molecular genetics of type I glycogen storage disease. *Trends Endocrinol Metab* 1999; **10**: 104.
 12. Van Creveld S. Glycogen storage disease. *Medicine* 1939; **18**: 1.
 13. Donnell GN. Growth in glycogen storage disease Type 1. Evaluation of endocrine function. *Am J Dis Child* 1969; **117**: 169.
 14. Howell RR, Ashton DM, Wyngaarden JB. Glucose-6-phosphatase deficiency glycogen storage disease. Studies on the interrelationships of carbohydrate lipid and purine abnormalities. *Pediatrics* 1962; **29**: 553.
 15. Spencer-Peet J, Norman ME, Lake BD *et al*. Hepatic glycogen storage disease. Clinical and laboratory findings in 23 cases. *Quart J Med New Series* 1971; **40**: 95.
 16. Hufton BR, Wharton BA. Glycogen storage disease (type 1) presenting in the neonatal period. *Arch Dis Child* 1982; **57**: 309.
 17. Lowery GH, Wilson JL. Observations on the treatment of a case of glycogen storage disease. *J Pediatr* 1949; **35**: 702.
 18. Melis D, Parenti G, Della Casa R *et al*. Brain damage in glycogen storage disease type I. *J Inherit Metab Dis* 2002; **25**(Suppl. 1): 248-P.
 19. Keller K, Schutz M, Podskarbi T *et al*. A new mutation of the glucose-6-phosphatase gene in a 4-year-old girl with oligosymptomatic glycogen storage disease type Ia. *J Pediatr* 1998; **132**: 360.
 20. Corby DG, Putnam CW, Greene HL. Impaired platelet function in glucose-6-phosphatase deficiency. *J Pediatr* 1974; **85**: 71.
 21. Fine RR, Kogut MB, Donnell GN. Intestinal absorption in type 1 glycogen storage disease. *J Pediatr* 1969; **75**: 632.
 22. Milla PJ, Atherton DA, Leonard JV *et al*. Disordered intestinal function in glycogen storage disease. *J Inherit Metab Dis* 1978; **1**: 155.
 23. Visser G, Rake JP, Kokke FTM *et al*. Intestinal function in glycogen storage disease type I. *J Inherit Metab Dis* 2002; **25**: 261.
 24. Michels VV, Beaudet AL. Hemorrhagic pancreatitis in a patient with glycogen storage disease type 1. *Clin Genet* 1980; **17**: 220.
 25. Zakon SJ, Oyamada A, Rosenthal IH. Eruptive xanthoma and hyperlipemia in glycogen storage disease (von Gierke's disease). *AMA Arch Derm Syph* 1953; **67**: 146.
 26. Hou J-W, Wang T-R, Tunnessen WW. Picture of the month. *Arch Pediatr Adolesc Med* 1996; **150**: 219.
 27. Fine RN, Wilson WA, Donnell GN. Retinal changes in glycogen storage disease type 1. *Am J Dis Child* 1968; **115**: 328.
 28. Alepa FP, Howell RR, Klinenberg JR, Seegmiller JE. Relationships between glycogen storage disease and tophaceous gout. *Am J Med* 1967; **42**: 58.
 29. Mason HH, Anderson DH. Glycogen disease of the liver (von Gierke's disease) with hepatomata. Case report with metabolic studies. *Pediatrics* 1955; **16**: 785.
 30. Alaupovic P, Fernandes J. The serum apolipoprotein profile of patients with glucose-6-phosphatase deficiency. *Pediatr Res* 1985; **19**: 380.
 31. Levy E, Thibault LA, Roy CC *et al*. Circulating lipids and lipoproteins in glycogen storage disease type 1 with nocturnal nasogastric feeding. *J Lipid Res* 1988; **29**: 215.
 32. Fernandes J, Pikaar NA. Ketosis in hepatic glycogenosis. *Arch Dis Child* 1972; **47**: 41.
 33. Binkiewicz A, Senior B. Decreased ketogenesis in von Gierke's disease (type 1 glycogenosis). *J Pediatr* 1973; **83**: 973.
 34. Dosman J, Crawhall JC, Klassen GA *et al*. Urinary excretion of C6-C10 dicarboxylic acids in glycogen storage diseases types 1 and 3. *Clin Chim Acta* 1974; **51**: 93.
 35. Jeune M, Francois R, Jarlot B. Contribution a l'étude des polycories glycoléniques du foie. *Rev Internat Hepatol* 1959; **9**: 1.
 36. Jakovcic S, Sorensen LB. Studies of uric acid metabolism in glycogen storage disease associated with gouty arthritis. *Arthritis Rheum* 1967; **10**: 129.
 37. Kelley WN, Rosenbloom FM, Seegmiller JE, Howell RR. Excessive production of uric acid in type 1 glycogen storage disease. *J Pediatr* 1968; **72**: 488.
 38. Lampert F, Mayer H, Tocci PM, Nyhan WL. Fanconi syndrome in glycogen storage disease. In: Nyhan WL (ed.). *Amino Acid Metabolism and Genetic Variation*. New York: McGraw Hill, 1967: 267.
 39. Garty R, Cooper M, Tabachnik E. The Fanconi syndrome associated with hepatic glycogenosis and abnormal metabolism of galactose. *J Pediatr* 1974; **85**: 821.
 40. Wolf B, Freehauf CL, Thomas JA *et al*. Markedly elevated serum biotinidase activity may indicate glycogen storage disease type Ia. *J Inherit Metab Dis* 2003; **26**: 805.
 41. Perkoff GT, Parker JV, Hahn RF. The effects of glucagons in three forms of glycogen storage disease. *J Clin Invest* 1962; **41**: 1099.
 42. Senior B, Loridan L. Functional differentiation of glycogenoses of the liver with respect to the use of glycerol. *N Engl J Med* 1968; **279**: 965.
 43. McAdams AJ, Hug G, Bove KE. Glycogen storage disease types I to X: criteria for morphologic diagnosis. *Hum Pathol* 1974; **5**: 463.
 44. Schoenheimer R. Über eine eigenartige Störung des Kohlenhydrat-Stoffwechsels. *Zeitschr Physiol Chem* 1929; **182**: 148.
 45. Illingworth B, Cori GT. Structure of glycogens and amylopectins. III. Normal and abnormal human glycogens. *J Biol Chem* 1952; **199**: 653.
 46. Kikuchi M, Hasegawa K, Handa I *et al*. Chronic pancreatitis in a child with glycogen storage disease type 1. *Eur J Pediatr* 1991; **150**: 852.
 47. Van Creveld S. Clinical course of glycogen storage disease. *Chem Weekblad* 1961; **57**: 445.
 48. Howell RR, Stevenson RE, Ben-Menachem Y *et al*. Hepatic adenomata in patients with type I glycogen storage disease (von Gierke's). *J Am Med Assoc* 1976; **236**: 1481.
 49. Limmer J, Fleig WE, Leupold D *et al*. Hepatocellular carcinoma in type I glycogen storage disease. *Hepatology* 1988; **8**: 531.
 50. Fink AS, Appelman HD, Thompson NW. Hemorrhage into a hepatic adenoma and type Ia glycogen storage disease: a case report and review of the literature. *Surgery* 1985; **97**: 117.

51. Miller JH, Gates GF, Landing BH *et al.* Scintigraphic abnormalities in glycogen storage diseases. *J Nucl Med* 1978; **19**: 354.
52. Restiano I, Kaplan BS, Stanley C, Baker L. Nephrolithiasis hypocitraturia and a distal renal tubular acidification defect in type I glycogen storage disease. *J Pediatr* 1993; **122**: 392.
53. Kikuchi M, Haginoya K, Miyabayashi S *et al.* Secondary amyloidosis in glycogen storage disease type Ib. *Eur J Pediatr* 1990; **149**: 344.
54. Weinstein DA, Somers MJ, Wolfsdorf JL. Decreased urinary citrate excretion in type 1a glycogen storage disease. *J Pediatr* 2001; **138**: 378.
55. Baker L, Dahlem S, Goldfarb S *et al.* Hyperfiltration and renal disease in glycogen storage disease type I. *Kidney Int* 1989; **35**: 1345.
56. Chen Y-T, Coleman RA, Scheinman JL *et al.* Renal disease in type I glycogen storage disease. *N Engl J Med* 1988; **318**: 7.
57. Reitsma-Bierens WCC, Smit GPA, Troelstra JA. Renal function and kidney size in glycogen storage disease type I. *Pediatr Nephrol* 1992; **6**: 236.
58. Beaudet AL, Anderson DC, Michels VV *et al.* Neutropenia and impaired neutrophil migration in type 1B glycogen storage disease. *J Pediatr* 1980; **97**: 906.
59. Schaub J, Haas JR. Glycogenosis type Ib complicated by severe granulocytopenia resembling inherited neutropenia. *Eur J Pediatr* 1981; **137**: 81.
60. Ambruso DR, McCabe ERB, Anderson D *et al.* Infectious and bleeding complications in patients with glycogenosis Ib. *Am J Dis Child* 1985; **139**: 691.
61. Roe TF, Thomas DW, Gilsanz V *et al.* Inflammatory bowel disease in glycogen storage disease type 1b. *J Pediatr* 1986; **109**: 55.
62. Visser G, Rake J-P, Fernandes J *et al.* Neutropenia neutrophil dysfunction and inflammatory bowel disease in glycogen storage disease type Ib: results of the European Study on Glycogen Storage Disease Type I. *J Pediatr* 2000; **137**: 187.
63. Kure S, Hou D-C, Suzuki Y *et al.* Glycogen storage disease type Ib without neutropenia. *J Pediatr* 2000; **137**: 253.
64. Kuijpers TW, Maiani NA, Tool AT *et al.* Apoptotic neutrophils in the circulation of patients with glycogen storage disease type 1b (GSD1b). *Blood* 2003; **101**: 5021.
65. Traisman AS, Traisman HS. Glycogen storage disease of the liver in siblings. *J Pediatr* 1953; **42**: 654.
66. Field JB, Drash AL. Studies in glycogen storage disease. II. Heterogeneity in the inheritance of glycogen storage disease. *Trans Assoc Am Phys* 1967; **80**: 284.
67. Golbus MS, Simpson TJ, Koresawa M *et al.* The prenatal determination of glucose-6-phosphatase activity by fetal liver biopsy. *Prenat Diagn* 1988; **8**: 401.
68. Harris RC, Olmo C. Liver and kidney glucose-6-phosphatase activity in children with normal and disease organs. *J Clin Invest* 1954; **33**: 1204.
69. Hers HG. Etudes enzymatiques sur fragments hépatiques; application a la classification des glycogenosis. *Rev Internat Hepatol* 1959; **9**: 35.
70. Waddell ID, Burchell A. Transverse topology of glucose-6-phosphatase in rat hepatic endoplasmic reticulum. *Biochem J* 1991; **275**: 133.
71. Burchell A. The molecular basis of the type I glycogen storage diseases. *Bioessays* 1992; **14**: 395.
72. Countaway JL, Waddell ID, Burchell A, Arion WJ. The phosphohydrolase component of the hepatic microsomal glucose-6-phosphatase system is a 365 kilodalton polypeptide. *J Biol Chem* 1988; **263**: 2673.
73. Illingworth B, Cori CF. Glucose-6-phosphatase and pyrophosphatase activities of homogenates of livers from patients with glycogen storage disease. *Biochem Biophys Res Commun* 1965; **19**: 10.
74. Hefferan PM, Howell RR. Genetic evidence for the common identity of glucose-6-phosphatase pyrophosphate-glucose phosphotransferase carbamyl phosphate-glucose phosphotransferase and inorganic pyrophosphatase. *Biochim Biophys Acta* 1977; **496**: 431.
75. Burchell A, Burchell B, Monaco M *et al.* Stabilization of glucose-6-phosphatase activity by a 21000 dalton hepatic microsomal protein. *Biochem J* 1985; **230**: 489.
76. Fulceri R, Bellamo G, Gamberucci A *et al.* Permeability of the rat liver microsomal membrane to glucose-6-phosphate. *Biochem J* 1992; **286**: 813.
77. Waddell ID, Hume R, Burchell A. A direct method for the diagnosis of human hepatic type Ib and Ic glycogen storage disease. *Clin Sci* 1989; **76**: 573.
78. Nordlie RC. Multifunctional glucose-6-phosphatase. Characteristics and function. *Cell Biol Life Sci* 1979; **24**: 2397.
79. Sann L, Matheiu M, Bourgeois J *et al.* In vivo evidence for defective activity of glucose-6-phosphatase in type Ib glycogenosis. *J Pediatr* 1980; **96**: 691.
80. Bashan N, Hagai Y, Potashnik R, Moses SW. Impaired carbohydrate metabolism of polymorphonuclear leukocytes in glycogen storage disease type Ib. *J Clin Invest* 1988; **81**: 1317.
81. Santer R, Schneppenheim R, Dombrowski A *et al.* Mutations in GLUT2 the gene for the liver-type glucose transporter in patients with Fanconi-Bickel syndrome. *Nat Genet* 1997; **17**: 324.
82. Stamm WE, Webb DI. Partial deficiency of hepatic glucose-6-phosphatase in an adult patient. *Arch Intern Med* 1975; **135**: 1107.
83. Burchell A, Waddell ID. The molecular basis of the genetic deficiencies of 5 of the components of the glucose-6-phosphatase system: improved diagnosis. *Eur J Pediatr* 1993; **152**(Suppl. 1): S18.
84. Lei K-J, Pan C-J, Shelly LL *et al.* Identification of mutations in the gene for glucose-6-phosphatase the enzyme deficient in glycogen storage disease type Ia. *J Clin Invest* 1994; **93**: 1994.
85. Akanuma J, Nishigaki T, Fujii K *et al.* Glycogen storage disease type 1a: molecular diagnosis of 51 Japanese patients characterization of splicing mutations by analysis of ectopically transcribed mRNA from lymphoblastoid cells. *Am J Med Genet* 2000; **91**: 107.
86. Annabi B, Hiraiwa H, Mansfield B *et al.* The gene for glycogen storage disease type Ib maps to chromosome 11q23. *Am J Hum Genet* 1998; **62**: 400.

87. Viegas-da-Cunha M, Gerin I, Chen Y-T *et al*. A gene on chromosome 11q23 coding for a putative glucose 6-phosphatase translocase is mutated in glycogen storage disease type Ib and type Ic. *Am J Hum Genet* 1998; **63**: 976.
88. Kure S, Suzuki Y, Matsubara Y *et al*. Molecular analysis of glycogen storage disease type Ib: identification of a prevalent mutation among Japanese patients and assignment of a putative glucose-6-phosphate translocase gene to chromosome 11. *Biochem Biophys Res Commun* 1998; **248**: 426.
89. Weston BW, Lin J-L, Muenzer J *et al*. Glucose-6-phosphatase mutation G188R confers an atypical glycogen storage disease type Ib phenotype. *Pediatr Res* 2000; **48**: 329.
90. Kishnani PS, Chuang TP, Bali D *et al*. Chromosomal and genetic alterations in human hepatocellular adenomas associated with type 1a glycogen storage disease. *Hum Mol Genet* 2009; **18**: 4781.
91. Folkman J, Philippart A, Tze WJ, Cirgler J. Portacaval shunt for glycogen storage disease. Value of prolonged intravenous hyperalimentation before surgery. *Surgery* 1972; **72**: 306.
92. Burr IM, O'Neill JA, Karzon DT *et al*. Comparison of the effects of total parenteral nutrition continuous intragastric feeding and portacaval shunt on a patient with type I glycogen storage disease. *J Pediatr* 1974; **85**: 792.
93. Greene HL, Slonim AE, Burr IM. Type I glycogen storage disease: a metabolic basis for advance in treatment. *Adv Pediatr* 1979; **26**: 63.
94. Greene HL, Slonim AE, O'Neill JA, Burr IM. Continuous nocturnal feeding for management of type I glycogen storage disease. *N Engl J Med* 1976; **294**: 423.
95. Perlman MB, Aker M, Slonim AE. Successful treatment of severe type glycogen storage disease with neonatal presentation by nocturnal intragastric feeding. *J Pediatr* 1979; **94**: 772.
96. Chen YT, Cornblath M, Sidbury JB. Cornstarch therapy in type I glycogen storage disease. *N Engl J Med* 1984; **310**: 171.
97. Chen Y-T, Bazarre C, Lee MM *et al*. Type I glycogen storage disease: nine years of management with cornstarch. *Eur J Pediatr* 1993; **152**(Suppl. 1): S56.
98. Chen Y-T, Scheinman JJ, Park HK *et al*. Amelioration of proximal renal tubular dysfunction in type I glycogen storage disease with dietary therapy. *N Engl J Med* 1990; **323**: 590.
99. Leonard JV, Dunger DB. Hypoglycaemia complicating feeding regimens for glycogen-storage disease. *Lancet* 1978; **12**: 1203.
100. Parker P, Burr I, Slonim A *et al*. Regression of hepatic adenomas in GSD-Ia with dietary therapy. *Gastroenterology* 1981; **81**: 534.
101. Greene HL, Slonim AE, Burr IM, Moran JR. Type I glycogen storage disease: five years of management with nocturnal intragastric feeding. *J Pediatr* 1980; **96**: 590.
102. Schroten H, Roesler J, Breidenbach T *et al*. Granulocyte and granulocyte-macrophage colony-stimulating factors for treatment of neutropenia in glycogen storage disease type Ib. *J Pediatr* 1991; **119**: 748.
103. Fernandes J, Alaupovic P, Wit JM. Gastric drip feeding in patients with glycogen storage disease type I: its effects on growth and plasma lipids and apolipoproteins. *Pediatr Res* 1989; **25**: 327.
104. Schwahn B, Rauch F, Wendel U, Schonau E. Low bone mass in glycogen storage disease type 1 is associated with reduced muscle force and poor metabolic control. *J Pediatr* 2002; **141**: 350.
105. Rake JP, Visser G, Labrune P *et al*. Guidelines for management of glycogen storage disease type 1. European study on glycogen storage disease type 1 (ESGSD 1). *Eur J Pediatr* 2002; **161**: S112.
106. Marti GE, Rick ME, Sidbury J, Gralnick HR. DDAVP infusion in five patients with type I glycogen storage disease and associated correction of prolonged bleeding times. *Blood* 1986; **68**: 180.
107. Emmett M, Narins RG. Renal transplantation in type I glycogenesis: failure to improve glucose metabolism. *J Am Med Assoc* 1978; **239**: 1642.
108. Malatack JJ, Iwatsuki S, Gartner JC *et al*. Liver transplantation for type I glycogen storage disease. *Lancet* 1983; **1**: 1073.
109. Kirschner BS, Baker AL, Thorp FK. Growth in adulthood after liver transplantation for glycogen storage disease type 1. *Gastroenterology* 1991; **101**: 238.

Glycogenosis type II/Pompe/lysosomal α -glucosidase deficiency

Introduction	438	Treatment	444
Clinical abnormalities	439	References	444
Genetics and pathogenesis	442		

MAJOR PHENOTYPIC EXPRESSION

Cardiomegaly and congestive cardiac failure, macroglossia, weakness of skeletal muscles, accumulation of glycogen in lysosomes of cardiac and skeletal muscle, and absence of acid α -1,4-glucosidase.

INTRODUCTION

Glycogenosis type II has been referred to as generalized glycogenosis because the defect is present in all cells. Clinical expression is most prominently manifested in the heart, and therefore the disease has been considered as cardiac glycogenosis. It was first described by Pompe [1] in an infant who had died of what had been called idiopathic cardiac hypertrophy. The sections stained with Best carmine identified the material as glycogen. This material was then found in a variety of other organs. Pompe recognized the possibility that a number of other patients diagnosed as having idiopathic cardiac hypertrophy might represent examples of glycogenosis, and he called attention to the report of vacuolization in the myocardium of such a patient previously reported by Sprague and his co-workers [2]. The tissue from this patient was then re-examined and found to contain glycogen [3]. In this family, a number of patients had died of cardiac disease in early infancy. A major impact on the understanding of this disease was made by Hers [4], who identified in lysosomes an α -glucosidase that was

active at acid pH and cleaved glycogen, as well as maltose (Figure 60.1). He further documented that this enzyme was deficient in tissues of patients with Pompe disease.

Pronunciation has become interesting in this disease. Many in the United States have pronounced it as if it were spelled pompy, while others more pedantic, such as the *New York Times* [5], have corrected us indicating that it should be pronounced like the city in Italy that was buried by the volcano. Actually, Pompe was an Austrian; the *Oxford English Dictionary* indicates it should be pronounced like ‘her’ or ‘bird’.

With the discovery of the enzyme defect, it soon became apparent that there were adult onset forms of the disease. In some patients, this is manifest only in exercise avoidance; others are wheelchair-bound and/or develop diaphragmatic failure.

These discoveries launched the field of lysosomal storage diseases. The gene for the enzyme has been localized to chromosome 17q25 [6]. It is now recognized that there are late onset purely myopathic forms of α -glucosidase deficiency and a spectrum of clinical phenotypes between

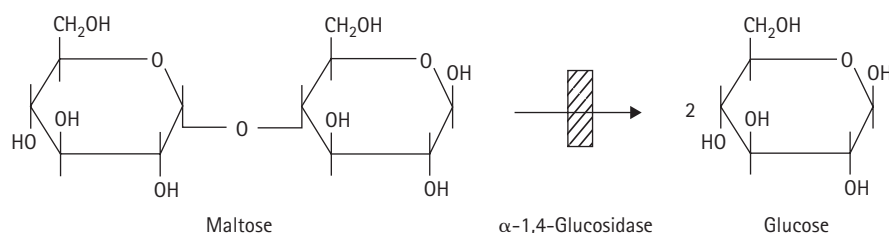


Figure 60.1 α -1,4-Glucosidase, the site of the defect in Pompe disease. This enzyme catalyzes the cleavage of other linear oligosaccharides, as well as of maltose.

that and the classic infantile Pompe disease [7]. Enzyme activity correlates generally with the degree of clinical severity. The gene has been cloned and a number of mutations has been identified [8]. A few have been identified with various ethnic groups [9, 10]. One mutation has accounted for a major proportion of Caucasian adult onset myopathic patients [11]. Enzyme replacement was first shown to be effective in quail [12].

CLINICAL ABNORMALITIES

The classic infantile form of glycogenosis II (Figures 60.2, 60.3, 60.4, 60.5, 60.6, 60.7, 60.8, and 60.9) is of rapidly progressive cardiomyopathy with massive cardiomegaly and death within the first year [13]. The discovery of the enzyme defect led to the recognition of a spectrum of variants. The clinical picture of those at the other end of the spectrum is that of adult-onset skeletal myopathy. Many variants have been observed between these two extremes.

In the infantile disease, manifestations begin in the first weeks or months of life [13, 14]. Symptoms may even be noted at birth. Poor feeding and failure to thrive may be early complaints, but cyanosis and attacks of dyspnea begin promptly, and there is rapid progression to intractable cardiac failure [15]. Death is by congestive failure, sometimes with a complicating terminal pneumonia.

Physical examination reveals signs of cardiac failure and the hallmark feature, cardiomegaly. Massive enlargement

of the heart is visible on roentgenograms (Figure 60.8). Significant cardiac murmurs are not usually present [16].

Hepatomegaly may be seen once cardiac failure begins, but it does not result from massive storage of glycogen in the liver. The electrocardiogram (Figure 60.9) may show very large QRS complexes in all leads and a short



Figure 60.3 AMS: An eight-month-old boy with Pompe disease. He was flaccid and intubated and had dilated cardiomyopathy.



Figure 60.2 A six-month-old infant with glycogenosis type II. She illustrates the severe hypotonia with which she presented. She also had cardiomyopathy and died at nine months of age of pneumonia.



Figure 60.4 AMS: The lips were thick and the tongue appeared large. The level of activity of α -glucosidase in fibroblasts was 3 percent of control. The V_{max} in muscle was markedly reduced.



Figure 60.5 AAD: A six-month-old infant with Pompe disease. Dyspnea and cyanosis began at two months. A sibling died at four months. The liver was palpable 4 cm below the costal margin. There was a grade I–III systolic cardiac murmur. Electrocardiogram (EKG) and echocardiogram revealed biventricular enlargement and poor left ventricular contraction.



Figure 60.7 The lips were full and the tongue quite large.



Figure 60.6 A girl with Pompe disease. The position was flaccid. She had a tracheostomy and required nasogastric feeding.



Figure 60.8 AK: A four-month-old infant with Pompe disease, had evidence of cardiomegaly on roentgenographic examination. (Courtesy of Dr MS Ahmad, Dharan Health Centre, Dharan, Saudi Arabia.)

PR interval [17]. Left axis deviation or an absence of the normal right axis deviation of this age and evidence of biventricular hypertrophy are seen, as well as T-wave changes and depression of the ST segment. Cardiac catheterization or echocardiogram shows biventricular hypertrophy and obstruction of left ventricular outflow [18]. Death within the first year of life is the usual course in

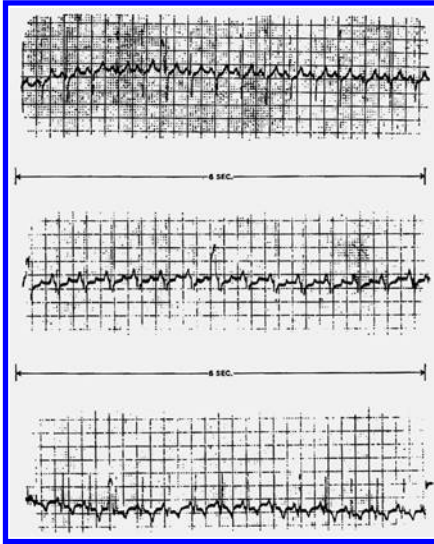


Figure 60.9 AL: The electrocardiogram (EKG) revealed biventricular hypertrophy. The leads shown, from the top, were 1, 2, and 3. (Courtesy of Dr MS Ahmad, Dharan Health Centre, Dharan, Saudi Arabia.)

this disease, but patients have been reported [19] in whom rapidly progressive cardiomegaly and cardiac failure led to death shortly after onset as late as 11 and 15 years.

Classification on the basis of age of onset appears less meaningful now that genetic variation can be expressed in terms of enzyme activity and the nature of mutation. In fact, atypical or nonclassic forms of the disease have been reported with onset in infancy [20]. Most of these infants had left ventricular hypertrophy, but they did not have left ventricular outflow obstruction. Death occurred later than the first year, often from myopathy-related pulmonary disease.

Skeletal muscle disease is prominent in all infantile



Figure 60.10 AAAD: The tongue appeared large and the lips thick. Biopsy of the right gastrocnemius revealed distention of the myocytes with stored glycogen. Activity of α -glucosidase was very low.

patients. It is manifested by marked hypotonia and weakness associated with diminished or absent deep tendon reflexes. The clinical picture may be suggestive of amyotonia congenita [21]. Muscle mass is normal, but the muscles may feel hard. Classically, the tongue is enlarged. A protuberant tongue (Figures 60.7 and 60.10) with failure to thrive, hypotonia, a protuberant abdomen and possibly an umbilical hernia may suggest a diagnosis of hypothyroidism or Down syndrome. Macroglossia is caused by infiltration of the muscle fibers of the tongue with glycogen, but macroglossia is noted in fewer than half of patients.

A small number of patients present in infancy or early childhood with a predominantly skeletal muscle disease without cardiac disease [22–24]. They may have lordosis or scoliosis, and may require surgical treatment. There may be localized pseudohypertrophy [25]. These patients display a more slowly progressive disease and death occurs by 19 years from pneumonia or respiratory failure.

A distinct group of patients has presented in the second or third decade with muscular weakness. This is the group that has been referred to as having adult onset disease (Figure 60.11). The clinical picture in these patients is that of a slowly progressive myopathy with little or no cardiac abnormality [23, 26–29]. One patient presented with unexplained hair loss [5]. In 12 patients with adult onset disease, four had ptosis [30]. This may be an alerting signal of the disorder. These patients may also die of respiratory failure [26]. A patient with this form of the disease was reported to develop a Wolff-Parkinson-White syndrome and a secondary atrioventricular block [31]. The early phenotype is that of a progressive proximal muscle weakness [32]. The legs and paraspinal muscles are particularly involved. Some patients present with back pain. Deep tendon reflexes may disappear. Urinary incontinence

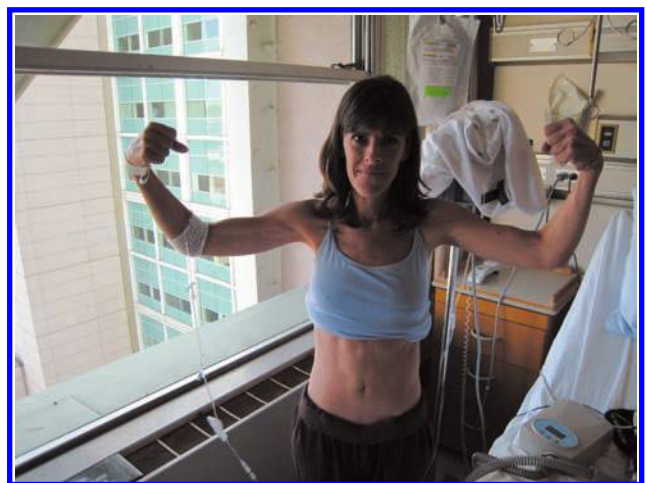


Figure 60.11 AB: A 45-year-old woman with Pompe disease. Her work that involved packing and lifting boxes led to impressive arm muscles, but she was already experiencing diaphragmatic weakness that she required assisted ventilation at least at night.

may indicate myopathy [33]. Diaphragmatic involvement may present as sleep apnea [34], but ultimately leads to respiratory failure or pneumonia.

Electromyography (EMG) in any of the forms of type II glycogenosis reveals pseudomyotonia and high frequency discharges and fibrillations [35]. Creatine kinase (CK) is usually elevated. It may be up to ten-fold in infantile patients. It may be normal, but its elevation can serve as the alerting signal for the diagnosis in a myopathic adult [36]. Transaminases may also be elevated [37]. Type II glycogenosis differs from the other glycogenoses in that no other abnormalities are detectable in the clinical chemistry laboratory. There is no hypoglycemia, and concentrations of lactic acid, uric acid, and lipids are normal.

The histopathology of this disease is one of generalized deposition of increased amounts of glycogen throughout the body, but without the enormous increase in storage that tends to increase massively the size of the liver in type I and type III. The material usually stains basophilic in hematoxylin and eosin, and red with special stains for glycogen (Figure 60.12). It is digestible with amylase and contains phosphates [38]. The typical lacework appearance of sections of the myocardium results from the deposition of glycogen in cardiac fibrils. In the electron microscopic picture (Figure 60.13), it is clear that the glycogen is membrane-bound, and that the accumulation is within the lysosome in contradistinction to the appearance of other glycogenoses [39]. However, in the muscle and in the heart of type II patients, there is also cytoplasmic accumulation of glycogen, and this has been correlated with destruction of contractile elements.

In addition to the accumulation in muscle and liver, deposition may be seen in motor nuclei in the brainstem and in the anterior horn cells of the spinal cord [22]. There

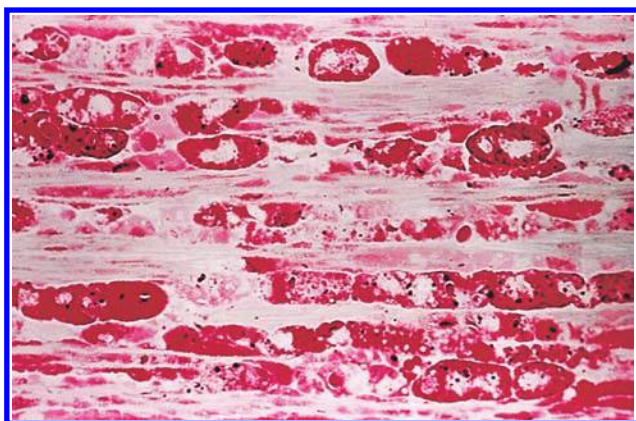


Figure 60.12 Periodic acid Schiff (PAS)-stained longitudinal section of muscle of a patient with Pompe disease. The infant presented as very floppy and had a severe deficiency of acid maltase. The red-staining accumulations of glycogen were dramatically visible. (Courtesy of Dr John S Romine, Department of Neurosciences, University of California, San Diego, La Jolla, California.)

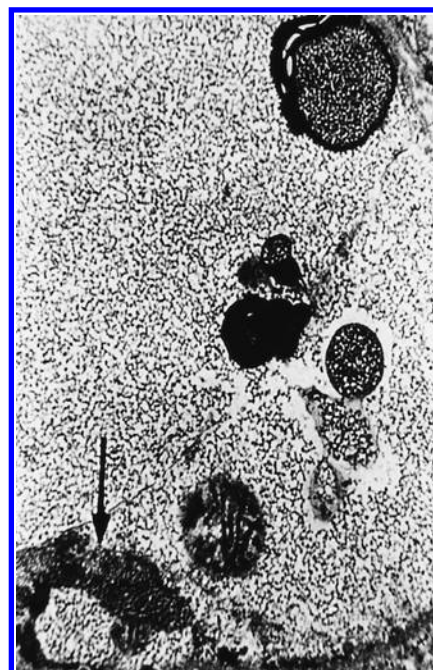


Figure 60.13 Electron micrograph of the muscle of the same baby demonstrates the extensive accumulation of glycogen. Most of the muscle fiber was replaced by glycogen granules. The arrow in the slide points to a remnant of a muscle fiber. Also evident are membrane-bound collections of glycogen. (Courtesy of Dr John S Romine, Department of Neurosciences, University of California, San Diego, La Jolla, California.)

is only slight deposition in the neurons of the cerebral cortex, but it may be seen even in fetal life [40]. Histologic examination of the adult onset patient, in whom the heart is clinically normal, may reveal no accumulation of glycogen in cardiac muscle, even though the enzyme defect is no different there than in skeletal muscle [41]. The histologic appearance of skeletal muscle of infantile and adult onset patients is indistinguishable [41]. Glycogen deposition may not be seen in the heart, liver, or brain of patients who have an adult onset. In classic infantile patients, the diagnosis may also be made by electron microscopic examination of biopsied skin [42], in which characteristic accumulation of glycogen within a lining membrane may be demonstrated.

GENETICS AND PATHOGENESIS

Type II glycogenosis is inherited in an autosomal recessive fashion. Consanguinity has been observed. Prevalence is high in Taiwan and South China; estimated frequency was 1 in 50,000 [43]. Many patients are seen in Saudi Arabia (Figures 60.2, 60.3, 60.4, 60.5, 60.6, 60.7, 60.8, 60.9, and 60.10). Elsewhere, the disease is rare. The molecular defect in type II glycogenosis is in α -1,4-glucosidase (Figure 60.1) [4]. The activity of this lysosomal enzyme in human liver at pH 4.0 is between three and ten times that at pH 7.4 [44]. It

is active against maltose and other oligosaccharides, as well as glycogen. The product is free-glucose [45]. The enzyme is normally widely distributed in tissues and it is present in fibroblasts, leukocytes, and amniocytes. In Pompe disease, deficiency of the enzyme has been demonstrable in all tissues measured [23, 28, 29] and this generalized deficiency is true of patients regardless of clinical phenotype. This explains the intralysosomal accumulation of glycogen in organs such as liver, where it would be inaccessible to other enzymes of glycogenolysis, such as phosphorylase and the debranching enzyme. In organs containing these enzymes, glycogen is not found outside the lysosomal fraction [46].

The diagnosis is best made by assay of the enzyme in muscle or fibroblasts [47]. The enzyme can be measured in transformed lymphoblasts or lymphocytes, but it may not be reliable in unfractionated leukocytes unless antibody against the enzyme is employed to prove that the activity being measured is not an unrelated glucosidase [48, 49]. Among groups of patients with different clinical phenotypes, there was an inverse correlation between the severity of clinical manifestation and the level of residual enzyme activity [50]. In general, the classic infantile patients display virtually absent activity, while adult onset patients usually have considerable residual activity, but there is appreciable overlap among patients in between. In the infantile presentation, a catalytically inactive, immunochemically reactive enzyme has been observed. In some patients with the adult phenotype, a reduction in the amount of enzyme protein has been reported [50–52]. Genetic heterogeneity is evident within both the infantile and adult forms of the disease; of nine patients with the infantile form, eight were cross-reacting material (CRM) negative and one was CRM positive [52]. In the adult phenotype, CRM-negative as well as CRM-positive variants have been observed. The enzyme undergoes extensive post-translational modification: seven N-linked glycosylations and phosphorylation of mannose moieties yield the mannose-6-phosphate lysosomal recognition marker.

All of the variants of glycogenosis type II described are inherited in an autosomal recessive fashion. Analysis of affected infants in reported kindreds after subtraction of the probands yielded a figure of 21.4 percent, approximating the 25 percent expected [53]. The incidence of consanguinity was high. Reduced activity in heterozygotes of α -1,4-glucosidase has been demonstrated by assessment of fibroblasts [54]. Heterozygote detection using leukocytes from peripheral blood has been accomplished, but it was unreliable [4]. An assay for heterozygosity was developed for lymphocytes stimulated by phytohemagglutinin [55]. Prenatal diagnosis has been carried out using cultivated amniocytes [56, 57]. Rapid prenatal diagnosis has been accomplished by electron microscopic examination of uncultured amniotic cells [58]. Of 26 fetuses at risk, six were found to be positive. Each of these prenatal diagnoses was confirmed by enzyme assay of amniocytes, and in tissues of three fetuses terminated and in three affected infants delivered. Prenatal

diagnosis has also been reported by assay of the enzyme in uncultured chorionic villus material [59].

The nature of the disease is usually quite similar in all affected members of a family. However, families have been reported in which there were examples of the typical infantile Pompe form of the disease and the late-onset adult [60, 61]. This situation has been shown to reflect allelic diversity [62] in which an affected grandparent with adult onset disease had two mutant alleles, one specifying partial deficiency and one complete. This second allele was passed to a son whose spouse also turned out to have such a gene, and an infantile classic patient was produced [61]. Somatic cell hybridization studies have failed to show evidence of complementation, and so a single locus appears to exist.

The gene has been localized to chromosome 17q25 [6]. The gene has been cloned and the sequence of the cDNA determined [63]. It contains 20 exons and is approximately 20 kb. Mutations have been defined in patients with different phenotypes. Among infantile patients, a majority of those studied have had undetectable mRNA [8, 64, 65]. A number of gross alterations in the gene have been found, such as deletion of exon 18 and stop codons [66, 67]. In contrast, a number of those with adult-onset phenotypes have had missense mutations [68]. Many of the mutations reported have been genetic compounds in which different mutations were on each allele. The number and variety of mutations observed indicate that the degree of heterogeneity in this population will be very great. Expression of a G-to-A transition in exon 11 *in vitro* indicated that the mutation coded for absence of catalytic activity [69]. This is consistent with the infantile onset phenotype. The common mutation in Chinese patients [10] is an Asp645Glu. The African mutation is an Arg854X [9]. Two deletions, del525T and del exon 18, are very common in Holland and in other Caucasian populations [70]. In 40 Italian patients with late onset disease, there were 26 different mutations, 12 novel [71]. The most common was IVS1A-AS-13T>G; it occurred on one allele in 34 patients. Among 98 Caucasian patients who were compound heterozygotes for this -13T>G mutation, there were a variety of second deleterious mutations, glucosidase activity was 3–20 percent of control and there was a wide spectrum of clinical phenotype [72]. Twelve different -13T>G haplotypes were observed. All of 22 Spanish patients with the -13T>G mutation were of the same haplotype, consistent with a founder effect [73].

The molecular biology of this gene is complicated by the fact that considerable polymorphism has been identified in individuals with no disease or enzymatic abnormality. Eleven restriction fragment length polymorphisms (RFLPs) have been identified which result from substitution of bases within introns [64], but silent mutations in the coding regions have also been observed [63]. These RFLPs may be useful for heterozygote detection and prenatal diagnosis. Prenatal diagnosis has been carried out in a family in which the mother carried a Δ T525 deletion and whose previous child had died of glycogenosis II [74]. Mutational analysis

correctly identified the absence of the mother's deletion in chorionic villus material, and enzyme analysis in the fetus was normal. However, it was concluded that enzyme analysis remains the method of choice because of the variety of mutations possible and the need to identify the mutation in each parent. If the mutation is known, this is a convenient method of prenatal diagnosis [75].

Newborn screening has been conducted in Taiwan since 2005 [76]. Activity of α -glucosidase was measured in spots of dried blood. In 13 patients, later onset Pompe disease was diagnosed and mutations were found. A confounding issue in diagnosis is the occurrence of patients with cardiomyopathy and lysosomal storage of glycogen in whom the activity of acid α -glucosidase is normal [76–78]. Arrhythmias, especially Wolff-Parkinson-White syndrome, were common in these patients. Most of those patients had impaired mental development.

TREATMENT

Supportive therapy, including ventilator assistance, is useful especially in advanced myopathic disease. Bone marrow transplantation was accomplished in cattle with α -glucosidase deficiency, but there was no effect on the disease [79].

Enzyme replacement therapy was employed early, using acid maltase purified from *Aspergillus* and human placenta, without clinical evidence of improvement. Recognition of the importance of the mannose-6-phosphate receptor-mediated lysosomal uptake of enzymes and the development of recombinant human enzyme have completely changed this area of investigation. Administration intravenously in acid glucosidase-deficient quail led to increased enzyme activity in muscle and a decrease in glycogen content to normal. Clinically, the birds righted themselves and even flew [12]. A clinical trial of recombinant human enzyme produced in rabbit milk was published [80], including evidence of improvement in muscle histopathology, decrease in cardiac size and improvement in function.

The modern era of treatment was ushered in by the infusion of recombinant human α -glucosidase in three infants with infantile disease. There was a decrease in cardiac size and maintenance of normal function for more than one year at report [81]. Enzyme replacement therapy has been reported to be effective, especially in infants [82]. Enzyme replacement therapy was summarized in 40 patients with infantile onset disease diagnosed between 1983 and 2008 in Taiwan [83]. There were five presymptomatic patients diagnosed by newborn screening. Regression of cardiomyopathy, lowering of B-type natriuretic peptide, and improved survival were recorded following treatment. Nevertheless, two died and two required ventilator support. Despite improvement in the electrocardiogram (EKG), three had life-threatening arrhythmias. None were in the newborn screening group.

In the late onset Pompe patients diagnosed by newborn screening [76], enzyme replacement therapy was initiated when symptoms or elevation of creatine kinase occurred. In a study of treatment in late onset Pompe disease, 90 patients were randomized 2:1 to enzyme replacement or placebo [84]. Cardiovascular abnormalities were identified, such as elevated left ventricular mass, a short PR interval, or decreased left ventricular function in 5–10 percent. There was no change in cardiac status in response to enzyme replacement therapy. In assessment of the metabolic myopathy the 6-minute walk test was used as a marker [85]. After 78 weeks of enzyme or placebo, there was significant improvement in walking distance, and stabilization of pulmonary function as measured by forced vital capacity (FVC). Mean increase in distance walked was 25 percent in the treatment group and 3.0 in the control.

REFERENCES

1. Pompe JC. Hypertrophie idiopathique du Coeur. *Ann Anat Pathol* 1933; **10**: 23.
2. Sprague HB, Cland EF, White PD. Congenital idiopathic hypertrophy of the heart. A case with unusual family history. *Am J Dis Child* 1931; **41**: 877.
3. Van Creveld S. Glycogen disease. *Medicine* 1939; **18**: 1.
4. Hers HG. α -Glucosidase deficiency in generalized glycogen storage disease (Pompe's disease). *Biochem J* 1963; **86**: 11.
5. Sanders L. The girl with unexplained hair loss. *New York Times* 2011; 16.
6. Solomon E, Swallow DM, Burgess S, Evan L. Assignment of the human acid α -glucosidase gene (α -GLU) to chromosome 17 using somatic cell hybrids. *Ann Hum Genet* 1979; **42**: 273.
7. Reuser AJJ, Kroos MMP, Hermans MMP *et al*. Glycogenosis type II (acid maltase deficiency). *Muscle Nerve* 1995; **53**: 61.
8. Martiniuk F, Mehler M, Tzall S *et al*. Extensive genetic heterogeneity in patients with acid α glucosidase deficiency as detected by abnormalities of DNA and mRNA. *Am J Hum Genet* 1990; **47**: 73.
9. Becker JA, Vlach J, Raben N *et al*. The African origin of the common mutation in African-American patients with glycogen storage disease type II (GSDII). *Am J Hum Genet* 1998; **62**: 991.
10. Shieh J-J, Lin C-Y. Frequent mutation in Chinese patients with infantile type of GSDII in Taiwan: evidence for a founder effect. *Hum Mutat* 1998; **11**: 306.
11. Kroos MA, Van Der Kraan M, Van Diggelen OP *et al*. Glycogen storage disease type II: frequency of three common mutant alleles and their associated clinical phenotypes studied in 121 patients. *Med Genet* 1995; **32**: 836.
12. Pennybacker M, Kikuchi T, Yang HW *et al*. Clinical and metabolic correction of Pompe disease by enzyme therapy in acid maltase-deficient quail. *J Clin Invest* 1998; **101**: 827.
13. di Sant'Agnese PA, Anderson DH, Mason HH, Bauman WA. Glycogen storage disease of the heart. I. Report of two cases in siblings with chemical and pathological studies. *Pediatrics* 1950; **6**: 402.

14. Gitzelmann R. Glukagonprobleme bei den Glykogenspeicherkrankheiten. *Helv Paediatr Acta* 1957; **21**: 425.
15. Pompe JC. Over idiopatische hypertrophie van het hart. *Ned Tijdschr Geneesk* 1932; **76**: 304.
16. Cottrill CM, Johnson GL, Noonan JA. Parental genetic contribution to mode of presentation in Pompe disease. *Pediatrics* 1987; **79**: 379.
17. Gillette PC, Nihill MR, Singer DB. Electrophysiological mechanisms for the short PR interval in Pompe disease. *Am J Dis Child* 1974; **128**: 622.
18. Seifert BL, Snyder MS, Klein AA *et al.* Development of obstruction to ventricular outflow and impairment of inflow in glycogen storage disease of the heart: serial echocardiographic studies from birth to death at 6 months. *Am Heart J* 1992; **123**: 239.
19. Antopol W, Boas EP, Levison W, Tuchman LR. Cardiac hypertrophy caused by glycogen storage disease in a 15-year-old boy. *Am Heart J* 1940; **20**: 546.
20. Slonim AE, Bulone L, Ritz S *et al.* Identification of two subtypes of infantile acid maltase deficiency. *J Pediatr* 2000; **137**: 283.
21. Clement DH, Godman GC. Glycogen disease resembling mongolism cretinism and amyotonia congenital. *J Pediatr* 1950; **36**: 11.
22. Gambetti P, Di Mauro S, Baker L. Nervous system in Pompe's disease: ultrastructure and biochemistry. *J Neuropathol Exp Neurol* 1971; **30**: 412.
23. Engel AG, Gomez MR, Seybold ME, Lambert EH. The spectrum and diagnosis of acid maltase deficiency. *Neurology* 1973; **23**: 95.
24. Tanaka K, Shimazu S, Oya N *et al.* Muscular form of glycogenosis type II (Pompe's disease). *Pediatrics* 1979; **63**: 124.
25. Iancu TC, Lerner A, Shiloh H *et al.* Juvenile acid maltase deficiency presenting as paravertebral pseudotumour. *Eur J Pediatr* 1988; **147**: 372.
26. Rosenow EC, Engel AE. Acid maltase deficiency in adults presenting as respiratory failure. *Am J Med* 1978; **64**: 485.
27. Engel AG. Acid maltase deficiency in adults: studies in four cases of a syndrome which may mimic muscular dystrophy or other myopathies. *Brain* 1970; **93**: 599.
28. Martin JJ, DeBarys T, den Tandt WR. Acid maltase deficiency in nonidentical adult twins: a morphological and biochemical study. *J Neurol* 1976; **213**: 105.
29. DiMauro S, Stern LZ, Mehler M *et al.* Adult onset acid maltase deficiency: a postmortem study. *Muscle Nerve* 1978; **1**: 27.
30. Groen WB, Leen WG, Vos AMC *et al.* Ptosis as a feature of late-onset glycogenosis type II. *Neurology* 2006; **67**: 2261.
31. Francesconi M, Auff E. Cardiac arrhythmias and the adult form of type II glycogenosis (letter). *N Engl J Med* 1982; **306**: 937.
32. Cinnamon J, Slonim AE, Black KS *et al.* Evaluation of the lumbar spine in patients with glycogen storage disease: CT demonstrations of patterns of paraspinal muscle atrophy. *Am J Neuroradiol* 1991; **12**: 1099.
33. Chancellor AM, Warlow CP, Webb JN *et al.* Acid maltase deficiency presenting with a myopathy and exercise-induced urinary incontinence in a 68-year-old male (letter). *J Neurol Neurosurg Psychiatry* 1991; **54**: 659.
34. Margolis ML, Howlett P, Goldgerg R *et al.* Obstructive sleep apnea syndrome in acid maltase deficiency. *Chest* 1994; **105**: 947.
35. Lenard HG, Schauab J, Keutel J, Osang M. Electromyography in type II glycogenosis. *Neuropediatric* 1974; **5**: 410.
36. Ausems MGEM, Lochman P, Van Diggelen OP *et al.* A diagnostic protocol for adult-onset glycogen storage disease type II. *Neurology* 1999; **52**: 851.
37. DiFiore MT, Manfredi R, Marri L *et al.* Elevation of transaminases as an early sign of late-onset glycogenosis type II (letter). *Eur J Pediatr* 1993; **152**: 784.
38. Martin JJ, DeBarys T, van Hoof F, Palladini G. Pompe's disease: an inborn lysosomal disorder with storage of glycogen: a study of brain and striated muscle. *Acta Neuropathol (Berl)* 1973; **23**: 229.
39. Baudhuin P, Hers HG, Loeb H. An electron microscopic and biochemical study of type II glycogenosis. *Lab Invest* 1964; **13**: 1139.
40. Hug G. Pre- and postnatal pathology enzyme treatment and unresolved issues in five lysosomal disorders. *Pharmacol Rev* 1979; **30**: 565.
41. Hug G. Glycogen storage diseases. *Birth Defects: Original Article Series* 1976; **12**: 145.
42. O'Brien JS, Bennett J, Veath L, Paa D. Lysosomal storage disorders. Diagnosis by ultrastructural examination of skin biopsy specimens. *Arch Neurol* 1975; **32**: 592.
43. Lin CY, Hwang B, Hsiao KJ, Yin YR. Pompe's disease in Chinese and the prenatal diagnosis by determination of alpha-glucosidase activity. *J Inherit Metab Dis* 1987; **10**: 11.
44. Hers HG, van Hoof F. Glycogen storage diseases: type II and type VI glycogenosis. In: Dickens F, Randle PJ, Whelan WJ (eds). *Carbohydrate Metabolism and Its Disorders*. New York: Academic Press, 1968: 151.
45. Hers HG. Glycogen storage disease. *Adv Metab Disord* 1964; **1**: 1.
46. Garancis JC. Type II glycogenosis. Biochemical and electron microscope study. *Am J Med* 1968; **44**: 289.
47. Angelini C, Engel AG, Titus JL. Adult acid maltase deficiency. Abnormalities in fibroblasts cultured from patients. *N Engl J Med* 1972; **287**: 948.
48. Koster JF, Slee RG, Hulsmann WC. The use of leukocytes as an aid in the diagnosis of a variant of glycogen storage disease type II (Pompe's disease). *Eur J Clin Invest* 1972; **2**: 467.
49. Dreyfus JC, Poënar L. Alpha glucosidases in white blood cells with reference to the detection of acid α -14-glucosidase deficiency. *Biochem Biophys Res Commun* 1978; **85**: 615.
50. Reuser AJJ, Koster JF, Hoogeveen A, Galjaard H. Biochemical immunological and cell genetic studies in glycogenosis type II. *Am J Hum Genet* 1978; **30**: 132.
51. Beratis NG, LaBadie GU, Hirschhorn K. Characterization of the molecular defect in infantile and adult acid alpha-glucosidase deficiency fibroblasts. *J Clin Invest* 1978; **62**: 1264.
52. Beratis NG, LaBadie GU, Hirschhorn K. Genetic heterogeneity in acid α -glucosidase deficiency. *Am J Hum Genet* 1983; **35**: 21.
53. Sidbury JB Jr. The genetics of the glycogen storage diseases. *Prog Med Genet* 1965; **IV**: 32.

54. Nitowsky HM, Grunfield A. Lysosomal α -glucosidase type II glycogenosis: activity in leukocytes and cell cultures in relation to genotype. *J Lab Clin Med* 1967; **69**: 472.
55. Hirschhorn K, Nadler HL, Waithe WI *et al*. Pompe's disease: detection of heterozygotes by lymphocytes stimulation. *Science* 1969; **166**: 1632.
56. Butterworth J, Broadhead DM. Diagnosis of Pompe's disease in cultured skin fibroblasts and primary amniotic fluid cells using 4-methylumbelliferyl- α -glycopyranoside as substrate. *Clin Chim Acta* 1977; **78**: 335.
57. Cox RP, Douglas G, Hutzler J *et al*. In-utero detection of Pompe's disease. *Lancet* 1970; **1**: 893.
58. Hug G, Soukup S, Ryan M, Chuck G. Rapid prenatal diagnosis of glycogen storage disease type II by electron microscopy of uncultured amniotic-fluid cells. *N Engl J Med* 1984; **310**: 1018.
59. Minelli A, Piantanida M, Simoni G *et al*. Prenatal diagnosis of metabolic diseases on chorionic villi obtained before the ninth week of pregnancy. *Prenat Diag* 1992; **12**: 959.
60. Koster JF, Busch HFM, Slee RG, van Weerden TW. Glycogenosis type II. The infantile and late-onset acid maltase deficiency observed in one family. *Clin Chim Acta* 1978; **87**: 451.
61. Loonen MCB, Busch HFM, Koster JF *et al*. A family with different clinical forms of acid maltase deficiency (glycogenosis type II): biochemical and genetic studies. *Neurology* 1981; **31**: 1209.
62. Hoefsloot LH, Van Der Ploeg AT, Kroos MA *et al*. Adult and infantile glycogenosis type II in one family explained by allelic diversity. *Am J Hum Genet* 1990; **46**: 45.
63. Martiniuk F, Mehler M, Tzall S *et al*. Sequence of the cDNA and 5' flanking region for human acid α glucosidase detection of an intron in the 5' untranslated leader sequences definition of 18 base pair polymorphisms and additional differences with previous cDNA and amino acid sequences. *DNA Cell Biol* 1990; **9**: 85.
64. Martiniuk F, Mehler M, Pellicer A *et al*. Isolation of a cDNA for human acid α glucosidase and detection of genetic heterogeneity for mRNA in three α glucosidase deficient patients. *Proc Natl Acad Sci USA* 1986; **83**: 9641.
65. Van der Ploeg AT, Hoefsloot LH, Hoogeveen-Westerveld M *et al*. Glycogenosis type II: protein and DNA analysis in five South African families from various ethnic origins. *Am J Hum Genet* 1989; **44**: 787.
66. Huie ML, Chen AS, Grix A *et al*. A de novo 13nt deletion a newly identified C647W missense mutation and a deletion of exon 18 in infantile onset glycogen storage disease type II (GSDII). *Hum Mol Genet* 1994; **3**: 1081.
67. Huie ML, Chen AS, Grix AW, Hirschhorn R. De novo mutation (13nt deletion) resulting in infantile GSDII (Pompe) in a child carrying a missense mutation on the other allele. *Am J Hum Genet* 1993; **53**(Suppl.): 906.
68. Hermans MMP, Kroos MA, de Graff E *et al*. Two mutations affecting the transport and maturation of lysosomal α -glucosidase in an adult case of glycogen storage type II. *Hum Mutat* 1993; **2**: 268.
69. Hermans MMP, de Graaff E, Kroos MA *et al*. Identification of a point mutation in the human lysosomal α glucosidase gene causing infantile glycogenosis type II. *Biochem Biophys Res Commun* 1991; **179**: 919.
70. Hirschhorn R, Huie ML. Frequency of mutations for glycogen storage disease type II in different populations: the del525T and delexon18 mutations are not generally 'common' in Caucasian populations. *J Med Genet* 1999; **36**: 85.
71. Montalvo ALE, Bembi B, Donnarumma M *et al*. Mutation profile of the GAA gene in 40 Italian patients with late onset glycogen storage disease type II. *Hum Mutat* 2006; **27**: 999.
72. Kroos MA, Pomponeo RJ, Hagemans ML *et al*. Broad spectrum of Pompe disease in patients with the same c.-32-13T-G haplotype. *Neurology* 2007; **68**: 110.
73. Gort L, Coll MJ, Chabas A. Glycogen storage disease type II in Spanish patients: high frequency of c.1076-1G-C mutation. *Mol Genet Metab* 2007; **92**: 183.
74. Kleijer WJ, van der Kraan M, Kroos MA *et al*. Prenatal diagnosis of glycogen storage disease type II: enzyme assay or mutation analysis? *Pediatr Res* 1995; **38**: 103.
75. Kroos MA, Waitfield AE, Joosse M *et al*. A novel acid α -glucosidase mutation identified in a Pakistani family with glycogen storage disease type II. *J Inher Metab Dis* 1997; **20**: 556.
76. Chien Y-H, Lee N-C, Huang H-J *et al*. Later-onset Pompe disease: early detection and early treatment initiation enabled by newborn screening. *J Pediatr* 2011; **158**: 1023.
77. Danon MJ, Oh SJ, Di Mauro S *et al*. Lysosomal glycogen storage disease with normal acid maltase. *Neurology* 1981; **31**: 51.
78. Byrne E, Dennett X, Crotty B *et al*. Dominantly inherited cardioskeletal myopathy with lysosomal glycogen storage and normal acid maltase levels. *Brain* 1986; **109**: 523.
79. Howell JM, Dorling PR, Shelton JN *et al*. Natural bone marrow transplantation in cattle with Pompe's disease. *Neuromusc Disord* 1991; **6**: 449.
80. Van den Hout H, Reuser AJJ, Vulto AG *et al*. Recombinant human α -glucosidase from rabbit milk in Pompe patients. *Lancet* 2000; **356**: 397.
81. Amalfitano A, Bengur AR, Morse RP *et al*. Recombinant human acid α -glucosidase enzyme therapy for infantile glycogen storage disease type II: results of a phase I/II clinical trial. *Genet Med* 2001; **3**: 132.
82. Bembi B, Cerini E, Danesino C *et al*. Management and treatment of glycogenosis type II. *Neurology* 2008; **71**: S12.
83. Chen L-R, Chen C-A, Chiu S-N *et al*. Reversal of cardiac dysfunction after enzyme replacement in patient with infantile-onset Pompe disease. *J Pediatr* 2009; **155**: 271.
84. Forsha D, Li JS, Smith PB *et al*. Cardiovascular abnormalities in late-onset Pompe disease and response to enzyme replacement therapy. *Genet Med* 2001; **13**: 2012.
85. Van der Ploeg AT, Clemens PR, Corzo D *et al*. A randomized study of α glucosidase alfa in late-onset Pompe's disease. *N Engl J Med* 2010; **362**: 1396.

Glycogenosis type III/amylo-1,6-glucosidase (debrancher) deficiency

Introduction	447	Treatment	452
Clinical abnormalities	448	References	453
Genetics and pathogenesis	451		

MAJOR PHENOTYPIC EXPRESSION

Hepatomegaly, hypoglycemia, late myopathy, storage of glycogen in liver and muscle, elevated transaminases and creatine phosphokinase, and deficient activity of the glycogen debranching enzyme amylo-1,6-glucosidase.

INTRODUCTION

The first patient described with glycogenosis type III was reported by Forbes in 1953 [1]. She had been noted at one year of age to have a large abdomen, and when she presented at 3.4 years, the liver was palpated at the left iliac crest. By 13 years, she was described as not appearing chronically ill [2]. The liver was studied by Illingworth and Cori [3, 4], who found the glycogen content of both muscle and liver to be increased. The structure of the glycogen was very abnormal and resembled a phosphorylase limit dextrin (Figure 61.1). The outer chains were abnormally short and the number of branch points was increased. The structure of the glycogen suggested that the defect was in the debrancher enzyme. This hypothesis was promptly confirmed by assay of the enzyme [4]. Activity of amylo-1,6-glucosidase was virtually absent in liver and muscle [4, 5]. The history of the disease is impressive in that the nature of the disorder and the enzyme defect were worked out in studies on the index case within a few years of the first report. The enzyme has two independent catalytic activities, glucosidase (EC 3.2.1.33) and transferase (EC 2.4.1.25). The cDNA has been cloned, and heterogeneity has been demonstrated in study of mRNAs [6]. The gene has been mapped to chromosome 1p21 [7].

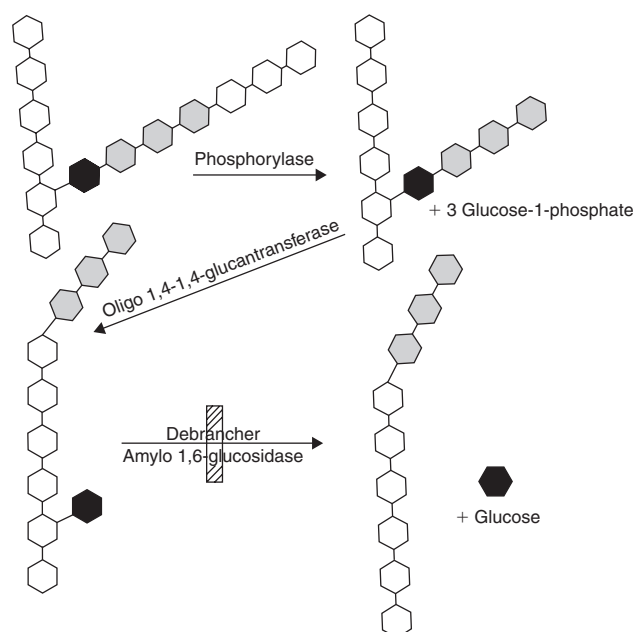


Figure 61.1 The sequential debranching of glycogen. Phosphorylase catalyzes the cleavage of glycosyl units in α -1,4 glucose unit before the amylo-1,6-glucosidase can cleave the glucose moiety in 1,6 linkage. This debrancher is the site of the defect in glycogenosis type III.



Figure 61.2 JL: An 18-month-old boy with glycogenosis type III [12] who presented with a history of increasing abdominal protuberance. He woke routinely at 4 a.m. for breakfast. (Illustration kindly provided by Dr Jon Wolff.)



Figure 61.4 SH: An infant with glycogenosis type III with protuberant abdomen.



Figure 61.3 JL: Close up of the abdomen highlighted the relatively enormous size of the liver. With time, he grew and the liver could only be found by palpation. (Illustration kindly provided by Dr Jon Wolff.)

CLINICAL ABNORMALITIES

The clinical manifestations of glycogenosis III tend to be milder than those of type I (Chapter 59), but the diseases cannot reliably be distinguished without laboratory procedures. Death in infancy has been recorded [2]. The most consistent clinical feature is hepatomegaly and it may be the only clinical abnormality at the time of presentation (Figures 61.2, 61.3, 61.4, 61.5, 61.6, and 61.7) [1]. In contrast to the patient with type I, these patients may also have some enlargement of the spleen [8]. The kidneys are not enlarged



Figure 61.5 MS: This infant with glycogenosis type III had a highly prominent abdomen.

in this disease; in fact, assessment of renal size by imaging of the abdomen may aid in distinguishing types I and III [9]. The enlargement of the liver may be massive; in infancy, it may interfere with walking or even standing. A two-year-old patient of ours simply toppled over if not supported in the standing position.

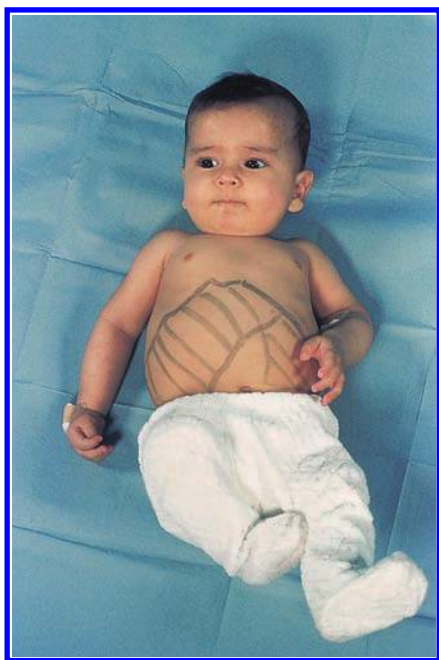


Figure 61.6 FMS: The enlarged liver created a very protruberant abdomen of this infant with glycogenosis type III.



Figure 61.7 Another girl with glycogenosis type III and marked hepatomegaly.

With time and growth, the patient's size tends to catch up with the liver which, while prominent, is less impressive. By adulthood, the abdomen usually appears normal [2]. The size of the liver may be normal by puberty [10].

Hypoglycemia is usually not a prominent feature of this disease, but fasting concentrations of glucose are



Figure 61.8 A five-year-old girl with glycogenosis type III. Unlike most patients with this disease, she had problems with hypoglycemia and was using an overnight glucose drip. Introduction of a cornstarch regimen at this time permitted its discontinuation.

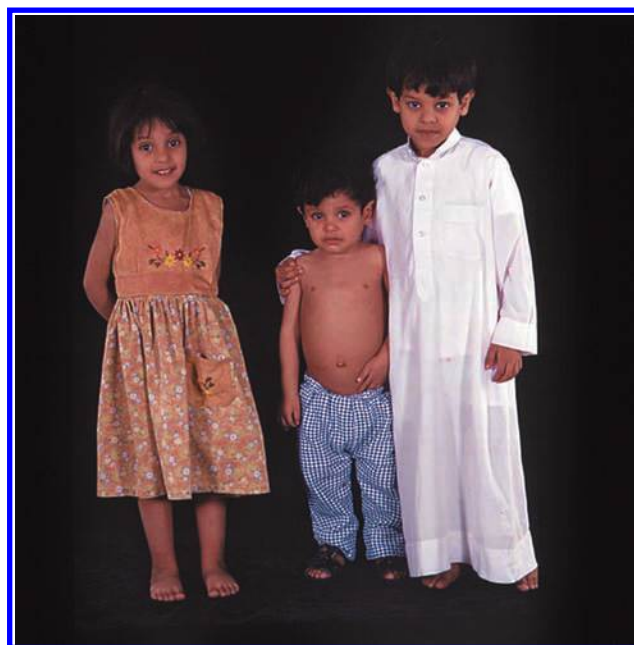


Figure 61.9 Three siblings with glycogenosis III who presented with hypoglycemia and elevated transaminases. Only the protruberant abdomen of the youngest suggests abnormality.

usually moderately reduced, and some patients, especially in infancy, have severe hypoglycemia (Figures 61.8 and 61.9) and even convulsions. Some patients have developed mental impairment [11], presumably as a consequence of

hypoglycemia. It is thought that hypoglycemia of infancy and early childhood may reflect developmental inadequacy of gluconeogenesis [9], but the behavior may also modulate the problem. For instance, one of our patients ate five meals a day and woke at 4 a.m. for breakfast [12]. In any case, by the second decade fasting hypoglycemia improves, and most adults with this disease tolerate fasting well [9]. Ketonuria may be observed after a moderate fast when the blood glucose approximates 40 mg/dL [13]. Hyperketonuria tends not to lead to metabolic acidosis or symptoms.

Vigorous catabolism of fatty acids and hyperactive fasting ketonuria are indicated by a ten-fold elevation in concentrations of 3-hydroxybutyric acid after a 12-hour fast. Concentrations of lipids may be elevated, but not to the degree seen in von Gierke disease [1, 14]. The concentration of cholesterol may be elevated in the absence of hypertriglyceridemia and there may be hyperbetalipoproteinemia [9]. Over the years, levels of lipids in the blood tend to decrease [1] and those of sugar to increase. Patients with type III disease do not develop xanthomas. Concentrations of uric acid are normal and levels of lactate are also usually normal. Concentrations of transaminases are usually elevated in infancy, and this may make the clinical picture confusing, suggesting hepatitis or hepatocellular insult as a cause of the hepatomegaly. On the other hand, the creatine kinase is usually elevated [12], so some of the enzyme levels could represent myopathy. Nevertheless, the histologic appearance of the liver usually indicates at least some fibrosis in this disease [8, 12]. Age-related change in transaminase activity has been observed, high in infancy and the first decade, followed by progressive reduction to adulthood [15]. Frank cirrhosis may be encountered, but cirrhosis does not usually progress with age [16, 17]. Cirrhosis appeared to be more common in Japan [18] and progressive cirrhosis and hepatic failure have been observed, particularly in that country [19–21]. Hepatic adenomas have been described [22], but they have not developed malignant change. On the other hand, hepatocellular carcinoma has been observed in end-stage cirrhosis [18, 21]. Ultrasound has been useful in following the progress of liver disease. Of 44 patients, by the third decade, no one had normal hepatic ultrasound [15]. Only two of these patients developed hepatic failure and required transplantation. Pallor has been described [23], but patients are usually not anemic.

Growth and development may be completely normal in this disease [1], but in some patients impairment of linear growth may be striking [12]. Many children are of normal size, although in a lower percentile for height than for weight and head circumference.

Renal tubular acidosis has been reported in two patients, along with severe failure to thrive [24]. One of the patients had a typical distal renal tubular acidosis, while the other had glycosuria and bicarbonate wasting, suggesting a Fanconi syndrome, as seen in type I glycogenosis (Chapter 59).

It has been recognized since the first patient that glycogen accumulates in muscle as well as liver. It has only more recently been recognized that this leads to a myopathy, especially by adulthood [25–27]. In fact, the late myopathy is the major morbidity of type III glycogenosis. There may be hypotonia, but muscle atrophy occurs as well as weakness. It is often notable in the interossei and over the thumbs. Atrophy in the legs has suggested diagnosis of Charcot–Marie–Tooth disease [28]. Weakness tends to be slowly progressive. Walking rapidly or upstairs brings out the weakness. Strenuous exercise cannot be effectively performed, but there is no tenderness, cramping, or myoglobinuria. Some patients have presented first in adulthood with progressive muscular weakness. Ultimately, the patient may be wheelchair-bound or bedridden. The electromyogram (EMG) may reveal a myopathic pattern with abundant fibrillations [26]. Nerve conduction may be abnormal. Activity of creatine kinase (CK) in serum is elevated [25] in the presence or absence of myopathic symptoms, but a normal level of CK does not rule out deficiency of debranching enzyme in muscle [29]. Deformity of the chest and kyphoscoliosis may be progressive. Some patients have had muscular fasciculations suggestive of motor neuron disease and peripheral nerve involvement has been documented in this disease [30]. Electron microscopic study of a 20-month-old boy revealed selective massive accumulation of glycogen in the Schwann cells of unmyelinated nerve fibers.

Cardiomyopathy may also occur; in fact, abnormalities of the electrocardiogram and echocardiographic evidence of biventricular hypertrophy are frequently observed, although rarely accompanied by cardiac symptoms [31, 32]. However, congestive cardiac failure has been described [26], as has exertional dyspnea and chest pain, and sudden death may occur [33].

Polycystic ovaries have been described in this disease, as in type I, but without effect on fertility [34].

Dysmorphic features, hypoplasia of the midface, depressed nasal bridge, broad up-turned nasal tips, indistinct filtral pillars, and bow-shaped lips have been described [35]. We do not see this in our patients. Histologic examination reveals the cells of the liver to be swollen and finely granular with an open nucleus. The material stored may be identified by Best stain as glycogen. Large vacuoles in the hepatocytes may be filled with periodic acid Schiff (PAS)-positive material. There is evidence of an increased amount of fibrous tissue within the lobules of the liver. In addition, there may be some proliferation of the bile ducts. Unlike the picture in glycogenosis type I, there is no infiltration with fat. Histologic examination of the muscle reveals abundant amounts of glycogen visible in subsarcolemmic areas of myofibrils [15]. The glycogen in this disease is more soluble than a normal glycogen, and therefore it tends to disappear more readily from conventional histologic preparations. Cryostat sections may be useful for biopsies. Electron microscopy reveals large pools of monoparticulate glycogen in hepatocytes

Table 61.1 Features by which type III glycogenosis may be distinguished from type I

Feature	Type III	Type I
Hypoglycemia	+	Severe
Bleeding diathesis	0	+
Splenomegaly	±	0
Enlarged kidneys	0	+
Myopathy	+	0
Elevated creatine kinase, transaminases	++	0
Fasting ketogenesis	++	+
Lactic acidemia	0	+
Alanine in plasma	Low	High
Hyperuricemia	0	+
Little or no response to glucagons after fast	+	+
Normal postprandial response to glucagon	+	0
Increase in blood glucose after galactose, fructose, or glycerol	+	0

[36]. Ovoid subunits measure 45–60 Å. In muscle, the glycogen accumulates beneath the sarcolemma. Glycogen is also seen between and within myofibrils. Glycogen may also be demonstrated by electron microscopy in granulocytes [36].

A variety of functional studies has been employed to document the presence of type III glycogenosis and to distinguish it from type I (Table 61.1). Among the most useful is the administration of glucagon after a 14-hour fast, following which there is little or no increase in blood glucose; and again 2–3 hours after a meal, following which there is a blood glucose response [37]; the rise in glucose may be normal, but it is usually reduced, although clearly present. This is consistent with the availability of glucose moieties on the elongated outer branches of glycogen to degradation of phosphorylase, even in the total absence of debranching activity. These patients also do not have lactic acidemia and, in particular contrast to patients with type I, the concentration of lactic acid does not increase after glucagon. Also in contrast to type I, there is a normal level of conversion of galactose, fructose, or glycerol to glucose [38].

The absence of highly elevated concentrations of lactic acid has been cited [39] as a reason why patients with this disease have been observed to have seizures at higher concentrations of glucose than those with type I disease, in whom the brain may be able to substitute lactate for glucose. On the other hand, seizures are not common in glycogenosis type III and concentrations of ketones in blood are elevated in this disease [40]. Mobilization of fat is very active, as is gluconeogenesis.

Metabolism of amino acids in glycogenosis type III is distinctly different from that of normal individuals and of patients with type I disease [41]. The major difference is in the responses of the principal gluconeogenic amino acid, alanine. The concentrations of alanine in plasma were significantly lower in 11 patients than in 27 controls [41]. This would be consistent with an overactive process of gluconeogenesis. In contrast in type I, concentrations of alanine are increased, consistent with defective gluconeogenesis. In type III, there were significantly lower concentrations of a number of other amino acids, notably threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, and lysine. This would be consistent with the operation of the alanine–glucose cycle in which branched-chain and other essential amino acids in muscle are depleted in order to serve as donors of nitrogen for the net synthesis of alanine from pyruvate in muscle [42]. Alanine is the only amino acid whose concentration in venous blood draining muscle is higher than arterial. The alanine formed is then transported through the blood to the liver, where it is converted to glucose.

Following the ingestion of glucose in a glucose tolerance test, concentrations of alanine in the blood rise dramatically in type III patients, while in type I patients they fall [41]. In controls, the level of alanine does not change after glucose. After a protein challenge with 4 g beef/kg, levels of alanine rose, but much less than in normals or than in type I patients and significantly less than the rise in alanine in type III after glucose. All of these data are compatible with an enhanced level of gluconeogenesis in type III disease.

The concentration of glycogen in the liver is markedly elevated. The amounts vary from 15 to 21 percent [10, 35]. Normal liver has less than 6 percent glycogen. Concentrations in muscle are less, approximating 6 percent in patients with type III disease and less than 1.5 percent in controls. Accumulation of glycogen of abnormal structure has also been reported in erythrocytes [43].

Depressed levels of enzymes other than the debranching enzyme are sometimes found in biopsies of these patients. For instance, glucose-6-phosphatase activities are sometimes somewhat low. That these are secondary effects has been suggested by the successful induction of increase to normal activity by the administration of triamcinolone [44].

GENETICS AND PATHOGENESIS

The disorder is transmitted by an autosomal recessive gene. Heterozygote detection has been carried out by the assay of debrancher enzyme activity in leukocytes and erythrocytes; intermediate levels were obtained [11, 45–47]. In families in which the patient lacks immunoprecipitable debrancher protein, carrier detection can be accomplished by Western blot [48].

The disorder has been recognized to be relatively high in frequency in Israel [11], where it makes up 73 percent

of patients with glycogen storage disease. All of these are Sephardic Jews of North African origin, in whom the incidence figure was estimated at one in 5420. Gene frequency also appears to be high in the Faroe islands [49]. Prenatal diagnosis has been accomplished by the study of the enzyme activity of immunoblot in cultured amniocytes and chorionic villus cells [50–52], but the assay of the enzyme is demanding, because activity is so low in these materials and immunoassay is only useful in families in which patients have no enzyme protein.

The molecular defect is in the activity of amylo-1,6-glucosidase (Figure 61.1) [4, 5, 53, 54]. A number of different methods has been used to assay the enzyme. The overall reaction catalyzes the production of glucose from phosphorylase limit dextrin. The partial reactions, transferase and glucosidase, appear to reside on a single polypeptide chain [55]. Enzyme deficiency has been demonstrated in leukocytes [45, 50, 56, 57], erythrocytes [58, 59], and cultured fibroblasts [60], as well as liver and muscle [4, 5, 53, 54]. In most patients, there is parallel reduction in all tissues tested, regardless of the assay method employed. However, there are some discrepancies, and it is clear that a few patients with proven hepatic deficiency of the enzyme have had normal activity in muscle, leukocytes, erythrocytes, or fibroblasts [6, 46, 48, 61–63]. This means that the diagnosis cannot be excluded without assay of the liver. It also seems likely that myopathy would only be expected in those with abnormal enzyme in muscle and this seems to be the case [29]. The most common situation in which activities of both glucosidase and transferase is deficient in both liver and muscle is sometimes referred to as IIIa; when the deficiency of both activities is found only in liver, it is referred to as IIIb; and the instances in which there is selective loss of only the glucosidase activity or only the transferase activity have been called IIIc and IIId, respectively [48, 54].

Studies using antibody to the normal debranching enzyme have revealed absence or considerable reduction of cross-reacting material (CRM) [48, 64]. The amounts of protein do not correlate with clinical severity [65].

The gene on chromosome 1p21 is in the area to which amylase genes have been mapped [7]. A variety of different mutations is responsible for this disease [6]. The isolation of the gene and determination of its sequence [6, 66] has elucidated a structure of 85 kb with 35 exons. The protein is a large monomeric structure of approximately 165 kDa. There is a single gene in liver and muscle, and the coding sequences of the two mRNAs are the same and code for a protein of 1532 amino acids. Isoforms differing in the 51 untranslated region appear to account for tissue differences.

Determination of the nature of mutation has provided correlations between molecular abnormality and clinical phenotype. In a patient with quite severe IIIa disease, an apparently homozygous mutation was found in which a single base (A4529) was inserted, changing a tyrosine to a stop codon at amino acid 1510 [67, 68]. Two mutations in exon 3 at amino acid codon 6, 17delAG and Q6X, were

found in three patients with type IIIb and not in type IIIa. Two patients had deletion of AG at nucleotides 17 and 18, leading to a truncated protein. The third had a C-to-T change at nucleotide 6, which changed glutamine to a stop. These mutations were not found in 31 patients with IIIa, two with IIId, or 28 controls. This permits DNA diagnosis on a blood sample in patients suspected of having type IIIb disease. A deletion, 4455delT, was found in all of the Sephardic Jewish patients [69]. A donor splice site mutation was found in a Japanese patient [70].

It has been observed that type IIIb patients have mutations in exon 3, while downstream mutations were found in IIIa patients [71]. In a series of 44 patients from Italy [15], two mutations were found in 69 percent, only one allele in 14 percent, and no mutations in 17 percent. Most mutations were null alleles. The IVS 21 + 1G/A intronic change was the most frequent (23 percent). Patients with null mutations tended to have more severe myopathic or hepatic disease.

Heterozygous expression of myopathy manifested by exercise induced myalgia and weakness was reported in a large family with a truncating mutation, p.W1327X [72].

A novel missense mutation p.R1147G was found in a Turkish study [73]. There were six nonsense mutations in this series. Mutations causing premature termination continue to be common in a variety of ethnic populations [74]. Genotype was reported to correlate for two mutations, one 3964 del T with severe, early onset symptoms, and the other an A to G transition at –12 upstream (IVS32-12A>G) associated with mild IIIb disease in quite different ethnic backgrounds [75].

TREATMENT

Frequent high carbohydrate feedings in infancy and early childhood are often all that is necessary for the management of the hypoglycemia of glycogenosis III. Cornstarch supplementation has facilitated this regimen, particularly if used at bedtime or during infant feedings at night [76–78]. Few, if any patients, need nocturnal enterogastric infusion, which may be dangerous if the tube becomes disconnected in a hyperinsulinemic state in the middle of the night. Serious, permanent brain damage may ensue. There is no need to restrict fructose and galactose in this disease.

A diet high in protein has been advocated for patients with this disease, but it has been our experience that pediatric patients will seldom eat a diet high enough in protein to make an appreciable difference in any of the abnormalities we could measure. Added protein has been obtained by mixing supplemental cornstarch with yogurt and other products [78].

The enhanced gluconeogenesis in this condition makes the use of high protein feedings logical [41]. Diets in which 20–25 percent of the calories are from protein and only 40–50 percent from carbohydrate have been employed. One approach has been to give between a quarter and a

third of the calories as nocturnal enteral therapy high in protein. This has been recommended for patients with myopathy or growth impairment [79, 80]. Patients were reported to experience improved muscle performance, in some cases quite dramatic. In others, effects were minimal or temporary [27]. In younger patients, there was improvement in growth [68, 78]. An older patient who discontinued the regimen experienced a recurrence of weakness that did not remit when therapy was resumed. Dramatic improvement in cardiomyopathy was reported [81] in a 22-year-old man on increasing the protein content of his diet to 30 percent of total calories.

In asymptomatic patients in childhood who nevertheless had elevated levels of CK and transaminases in the blood, we found no effects of cornstarch or a high protein diet on these abnormalities. In contrast, we reasoned that if muscle were being broken down to provide alanine for gluconeogenesis, the provision of supplemental alanine might be therapeutic. We have observed a considerable improvement in levels of CK and transaminases [82]. Doses of alanine have ranged from 0.25 to 2.0 g/kg per day. A teaspoonful of alanine weighs 3.78 g.

REFERENCES

- Forbes GB. Glycogen storage disease. Report of a case with abnormal glycogen structure in liver and skeletal muscle. *Pediatrics* 1953; **42**: 645.
- Recant L. Recent developments in the field of glycogen metabolism and in the diseases of glycogen storage. *Am J Med* 1955; **19**: 610.
- Illingworth B, Cori GT. Structure of glycogens and amylopectins. III Normal and abnormal human glycogens. *J Biol Chem* 1952; **199**: 653.
- Illingworth B, Cori GT, Cori CF. Amylo-16-glucosidase in muscle tissue in generalized glycogen storage disease. *J Biol Chem* 1956; **218**: 123.
- Hers HG. Etudes enzymatiques sur fragments hépatiques; application à la classification des glycogénoses. *Rev Int Hepatol* 1959; **9**: 35.
- Yang B-Z, Ding J-H, Enghild JJ *et al*. Molecular cloning and nucleotide sequence of cDNA encoding human muscle glycogen debranching enzyme. *J Biol Chem* 1992; **267**: 9294.
- Yang-Zeng TL, Zheng K, Yu J *et al*. Assignment of the human glycogen debrancher gene to chromosome 1p21. *Genomics* 1992; **13**: 931.
- Brandt IK, De Luca VA Jr. Type III glycogenosis: a family with an unusual tissue distribution of the enzyme lesion. *Am J Med* 1966; **40**: 779.
- Moses SW. Pathophysiology and dietary treatment of the glycogen storage diseases. *J Pediatr Gastroenterol Nutr* 1990; **11**: 155.
- Brown BI, Brown DH. The glycogen storage diseases: types I, III, IV, V, VII, and unclassified glycogenoses. In: Whelan WJ (ed.). *Carbohydrate Metabolism and Its Disorders*, vol. 2. New York: Academic Press, 1968: 123.
- Levin S, Moses SW, Chayoth R *et al*. Glycogen storage disease in Israel. A clinical biochemical and genetic study. *Israel J Med Sci* 1967; **3**: 297.
- Nyhan WL, Sakati NO. Glycogenosis type III; amylo-16-glucosidase (debrancher) deficiency. In: Nyhan WL, Sakati NO (eds). *Diagnostic Recognition of Genetic Disease*. Philadelphia: Lea and Febiger, 1987: 205.
- Von Buerhrdel P, Boehme HJ, Hubald J. Metabolische adaptation im Nahrungskarenztest bei Kinder mit Glykogenosen der Typen III und VI. *Kinderarztl Prax* 1987; **55**: 543.
- Vitek B, Srachova D, Toma M *et al*. Hyperlipidemia in type III glycogenosis. *Acta Paediatr Scand* 1970; **59**: 701.
- Lucchiara S, Santoro D, Pagliarini S *et al*. Clinical, biochemical and genetic features of glycogen debranching enzyme deficiency. *Acta Myol* 2007; **26**: 72.
- Starzl TE, Putnam CW, Portern KA *et al*. Portal diversion for the treatment of glycogen storage disease in humans. *Ann Surg* 1973; **178**: 525.
- Fellows IW, Lowe JS, Ogilvie AL *et al*. Type III glycogenosis presenting as liver disease in adults with atypical histological features. *J Clin Pathol* 1983; **36**: 431.
- Momoi T, Sano H, Yamanaka C *et al*. Glycogen storage disease type III with muscle involvement. Reappraisal of phenotypic variability and prognosis. *Am J Med Genet* 1992; **42**: 696.
- Rosenfeld EL, Popova IA, Chibisov IV. Some cases of type III glycogen storage disease. *Clin Chim Acta* 1976; **67**: 123.
- Markowitz A, Chen Y-T, Muenzer J *et al*. A man with type III glycogenosis associated with cirrhosis and portal hypertension. *Gastroenterology* 1993; **105**: 1882.
- Haagsma E, Smit G, Niezen-Koning K *et al*. Type IIIb glycogen storage disease associated with end-stage cirrhosis and hepatocellular carcinoma. The Liver Transplant Group. *Hepatology* 1997; **25**: 537.
- Labrune P, Trioche P, Duvaltier I *et al*. Hepatocellular adenomas in glycogen storage disease type I and III: a series of 43 patients and review of the literature. *J Pediatr Gastroenterol Nutr* 1997; **24**: 276.
- Spencer-Peet J, Norman ME, Lake BD *et al*. Hepatic glycogen storage disease. Clinical and laboratory findings in 23 cases. *Quart J Med* 1971; **40**: 95.
- Chen J, Friedman M. Renal tubular acidosis associated with type III glycogenosis. *Acta Paediatr Scand* 1979; **68**: 779.
- Brunberg JA, McCormick WF, Schochet SS. Type III glycogenosis: an adult with diffuse weakness and muscle wasting. *Arch Neurol* 1971; **25**: 171.
- DiMauro S, Hartwig GB, Hays A *et al*. Debrancher deficiency: neuromuscular disorder in 5 adults. *Ann Neurol* 1976; **5**: 422.
- Moses SW, Gadoth N, Bashan N *et al*. Neuromuscular involvement in glycogen storage disease type III. *Acta Paediatr Scand* 1986; **75**: 289.
- Cornelio F, Bresolin N, Singer PA *et al*. Clinical varieties of neuromuscular disease in debrancher deficiency. *Arch Neurol* 1984; **41**: 1027.
- Coleman RA, Winter HS, Wolf B *et al*. Glycogen storage disease type III (glycogen debranching enzyme deficiency: correlation of biochemical defects with myopathy and cardiomyopathy). *Ann Intern Med* 1992; **116**: 896.

30. Powell HC, Haas R, Hall CL *et al*. Peripheral nerve in type III glycogenosis: selective involvement of unmyelinated fiber Schwann cells. *Muscle Nerve* 1985; **8**: 667.
31. Moses SW, Wanderman KL, Myroz A, Friedman M. Cardiac involvement in glycogen storage disease type III. *Eur J Pediatr* 1989; **431**: 1.
32. Olson LJ, Reeder GS, Noller KL *et al*. Cardiac involvement in glycogen storage disease. III: Morphologic and biochemical characterization with endomyocardial biopsy. *Am J Cardiol* 1984; **53**: 980.
33. Miller CG, Alleyne GA, Brooks SEH. Gross cardiac involvement in glycogen storage disease type III. *Br Heart J* 1972; **34**: 862.
34. Lee P, Patel A, Hindmarsh P *et al*. The prevalence of polycystic ovaries in the hepatic glycogen storage diseases: its association with hyperinsulinism. *Clin Endocrinol* 1995; **42**: 601.
35. Cleary MA, Walter JH, Kerr BA *et al*. Facial appearance in glycogen storage disease type III. *Clin Dysmorph* 2002; **11**: 117.
36. Garancis JC, Panares RR, Good TA, Kuzman JF. Type III glycogenosis A biochemical and electron microscopic study. *Lab Invest* 1970; **22**: 468.
37. Perkoff GT, Parker VJ, Hahan RE. The effects of glucagons in three forms of glycogen storage disease. *J Clin Invest* 1962; **41**: 1099.
38. Senior B, Loridan L. Studies of liver glycogenosis with particular reference to the metabolism of intravenously administered glycerol. *N Engl J Med* 1968; **279**: 958.
39. Huijing F. Glycogen metabolism and glycogen-storage diseases. *Physiol Rev* 1975; **55**: 609.
40. Fernandes J, Pikaar NA. Ketosis in hepatic glycogenosis. *Arch Dis Child* 1972; **47**: 41.
41. Slonim AE, Coleman RA, Moses S *et al*. Amino acid disturbances in type III glycogenosis: differences from type I glycogenosis. *Metabolism* 1983; **32**: 70.
42. Odessey R, Kairallath EA, Goldberg AL. Origin and possible significance of alanine production by skeletal muscle. *J Biol Chem* 1974; **249**: 7623.
43. Sidbury JB Jr, Cornblath M, Fisher J, House E. Glycogen in erythrocytes of patients with glycogen storage disease. *Pediatrics* 1961; **27**: 103.
44. Moses SW, Leven S, Chayoth R, Steinitz K. Enzyme induction in a case of glycogen storage disease. *Pediatrics* 1966; **38**: 111.
45. Williams HE, Kendig EM, Field JB. Leukocyte debranching enzyme in glycogen-storage disease. *J Clin Invest* 1963; **42**: 656.
46. Williams C, Field JB. Studies in glycogen-storage disease: limit dextrinosis a genetic study. *J Pediatr* 1968; **72**: 214.
47. Shin YS, Ungar R, Rieth M, Endres W. A simple assay for amylo-16-glucosidase to detect heterozygotes for glycogenosis type III in erythrocytes. *Clin Chem* 1984; **30**: 717.
48. Ding J-H, de Barsey T, Brown BI *et al*. Immunoblot analyses of glycogen debranching enzyme in different subtypes of glycogen storage disease type III. *J Pediatr* 1990; **116**: 95.
49. Cohn J, Wang P, Hauge M *et al*. Amylo-16-glucosidase deficiency (glycogenosis type III) in the Faroe Islands. *Hum Hered* 1975; **25**: 115.
50. Yang B-Z, Ding J-H, Brown BI, Chen Y-T. Definitive prenatal diagnosis for type III glycogen storage disease. *Am J Hum Genet* 1990; **47**: 735.
51. Maire I, Mandon G, Mathieu M. First trimester prenatal diagnosis of glycogen storage disease type III. *J Inher Metab Dis* 1989; **12**(Suppl. 2): 292.
52. Van Diggelen OP, Janse HC, Smit GPA. Debranching enzyme in fibroblasts amniotic fluid cells and chorionic villi: pre- and postnatal diagnosis of glycogenosis type III. *Clin Chim Acta* 1985; **149**: 129.
53. Hers HG, Verhue W, Van Hoof F. The determination of amylo-16-glucosidase. *Eur J Biochem* 1967; **2**: 257.
54. Hoof F, Hers HG. The subgroups of type III glycogenosis. *Eur J Biochem* 1967; **2**: 265.
55. Lee EYC, Smith EE, Whelan WJ. Glycogen and starch debranching enzymes. *Enzymes* 1971; **5**: 191.
56. Huijing F. Amylo-16-glucosidase activity in normal leukocytes and in leukocytes of patients with glycogen storage disease. *Clin Chim Acta* 1964; **9**: 269.
57. Steinitz K, Bodur H, Arman T. Amylo-16-glucosidase activity in leukocytes from patients with glycogen-storage disease. *Clin Chim Acta* 1963; **8**: 807.
58. Chayoth R, Moses SW, Steinitz K. Debrancher enzyme activity in blood cells of families with type III glycogen storage disease. *Israel J Med Sci* 1967; **3**: 422.
59. Van Hoof F. Amylo-16-glucosidase activity and glycogen content of the erythrocytes of normal subjects patients with glycogen-storage disease and heterozygotes. *Eur J Biochem* 1967; **2**: 271.
60. Justice P, Ryan C, Hsia DY, Krmpotik E. Amylo-16-glucosidase in human fibroblasts: studies in type III glycogen-storage disease. *Biochem Biophys Res Commun* 1970; **39**: 301.
61. Deckelbaum RJ, Russell A, Shapira E *et al*. Type III glycogenosis: atypical enzyme activities in blood cells in two siblings. *J Pediatr* 1972; **81**: 955.
62. Gutman A, Barash V, Schramm H *et al*. Incorporation of (¹⁴C) glucose into alpha-14-bonds of glycogen by leukocytes and fibroblasts of patients with type III glycogen storage disease. *Pediatr Res* 1985; **19**: 218.
63. Brown BI. Diagnosis of glycogen storage disease. In: Wapnir RA (ed.). *Congenital Metabolic Disease Diagnosis and Treatment*. Basel: Marcel Dekker, 1985: 227.
64. Dreyfus JC, Alexandre Y. Immunological studies on glycogen-storage disease type III and V. Demonstration of the presence of an immunoreactive protein in one case of muscle phosphorylase deficiency. *Biochem Biophys Res Commun* 1971; **44**: 1364.
65. Yang B-Z, Stewart C, Ding J-H, Chen Y-T. Type III glycogen storage disease: an adult case with mild disease but complete absence of debrancher protein. *Neuromusc Dis* 1991; **1**: 173.
66. Liu W, de Castro ML, Takrama J *et al*. Molecular cloning sequencing and analysis of the cDNA for rabbit muscle glycogen debranching enzyme. *Arch Biochem Biophys* 1993; **306**: 1.
67. Yang B-Z, Ding J-H, Bao Y *et al*. Molecular basis of the enzymatic variability in type III glycogen storage disease (GSD-III). *Am J Hum Genet* 1992; **51**: A28.
68. Shen J, Bao Y, Chen Y-T. A nonsense mutation due to a single base insertion in the 39-coding region of glycogen debranching enzyme gene is associated with a severe phenotype in a patient with GSD type IIIa. *Hum Mutat* 1997; **9**: 37.

69. Parvar R, Moses S, Shen J *et al*. A single base deletion in the 39 coding region of glycogen debranching enzyme gene is prevalent in glycogen storage disease type IIIa in a population of North African Jewish patients. *Eur J Hum Genet* 1998; **5**: 266.
70. Okubo M, Aoyama Y, Murase T. A novel donor splice-site mutation in the glycogen debranching enzyme gene is associated with glycogen storage disease type III. *Biochem Biophys Res Commun* 1996; **255**: 695.
71. Shen J, Bao Y, Liu HM *et al*. Mutations in exon 3 of the glycogen storage disease type III that is differentially expressed in liver and muscle. *J Clin Invest* 1996; **98**: 352.
72. Schoser B, Gläser D, Muller-Höcker J. Clinicopathological analysis of the homozygous p.W132X AGL mutation in glycogen storage disease type 3. *Am J Med Genet A* 2008; **146A**: 2911.
73. Aoyama Y, Ozer I, Demirkol M *et al*. Molecular features of 23 patients with glycogen storage disease type III in Turkey: novel mutation p.R1147G associated with isolated glucosidase deficiency, along with 9 AGL mutations. *J Hum Genet* 2009; **54**: 681.
74. Endo Y, Horinishi A, Vorgerd M *et al*. Molecular analysis of the AGL gene: heterogeneity of mutations in patients with glycogen storage disease type III from Germany, Canada, Afghanistan, Iran, and Turkey. *J Hum Genet* 2006; **51**: 958.
75. Shaiu WL, Kishnani PS, Shen J *et al*. Genotype-phenotype correlation in two frequent mutations and mutation update type III glycogen storage disease. *Mol Genet Metab* 2000; **69**: 16.
76. Borowitz SM, Green HL. Cornstarch therapy in a patient with type III glycogen storage disease. *J Pediatr Gastroenterol Nutr* 1987; **6**: 631.
77. Gremse DA, Bucuvals JC, Balistreri WF. Efficacy of cornstarch therapy in type III glycogen-storage disease. *Am J Clin Nutr* 1990; **52**: 671.
78. Ullrich K, Schmidt H, van Teeffelen-Heithoof A. Glycogen storage disease type I and III and pyruvate carboxylase deficiency: results of long-term treatment with uncooked cornstarch. *Acta Paediatr Scand* 1988; **77**: 531.
79. Slonim AE, Weisberg C, Benke P *et al*. Reversal of debrancher deficiency myopathy by the use of high protein nutrition. *Ann Neurol* 1982; **11**: 420.
80. Slonim AE, Coleman RA, Moses WS. Myopathy and growth failure in debrancher enzyme deficiency: improvement with high-protein nocturnal enteral therapy. *J Pediatr* 1984; **105**: 906.
81. Dagli AI, Zori RT, McCune H *et al*. Reversal of glycogen storage disease type IIIa-related cardiomyopathy with modification of diet. *J Inherit Metab Dis* 2009; DOI: 10.1007/s10545-009-1088-x (on line).
82. Nyhan WL, Rice-Asaro M, Acosta P. Advances in the treatment of amino acid and organic acid disorders. In: Desnick RJ (ed.). *Treatment of Genetic Diseases*. New York: Churchill Livingstone, 1991: 45.

PEROXISOMAL DISORDERS

62.	Adrenoleukodystrophy	459
63.	Neonatal adrenoleukodystrophy/disorders of peroxisomal biogenesis	469

Adrenoleukodystrophy

Introduction	459	Treatment	464
Clinical abnormalities	460	References	465
Genetics and pathogenesis	463		

MAJOR PHENOTYPIC EXPRESSION

X-linked cerebral demyelinating disease with onset in males in childhood, usually with behavioral abnormalities progressive to dementia, speech difficulty, and loss of vision and hearing; relentless progression to decorticate spastic quadriparesis; pigmentation of the skin; adrenal insufficiency; cytoplasmic inclusions; accumulation of very long-chain fatty acids, particularly hexacosanoate (c26:0); defective activity of very long-chain acylCoA synthetase; and abnormal adenosine triphosphate-(ATP)-binding cassette (ABCD1) peroxisomal transmembrane transporter protein.

INTRODUCTION

Adrenoleukodystrophy (ALD) is a progressive cerebral degenerative disorder with onset in childhood, in which there is increased pigmentation of the skin and laboratory evidence of degenerative disease of the adrenals [1, 2]. The disease appears to have been first described in 1910 by Haberland and Spieler [3], and the neuropathological findings by Schilder [4]. In 1923, Siemerling and Creutzfeld [5] were the first to put together the adrenal and cerebral disease in the definitive description; they referred to it as a bronzed disease in which there was sclerosis and encephalomyelitis, and it has been referred to as a bronzed Schilder disease, but in spite of the pigment, which may serve as the alerting sign to the diagnosis, most patients have full progression of the cerebral manifestations without the clinical symptomatology of adrenal insufficiency [2, 6, 7]. The lipid inclusions in the adrenal were first recognized by Schaumberg and colleagues [6, 7] who found that they were composed of cholesterol esters [8]. The term 'adrenoleukodystrophy' was first employed by Blaw [9]. This disease appears to be the cause of Schilder disease in most males [9].

Neonatal adrenoleukodystrophy (Chapter 63) is a very different disease with an entirely different phenotype and an autosomal recessive, as opposed to X-linked transmission.

The X-linked nature of the disease was first recognized by Fanconi and colleagues [10]. A more indolent, slowly

progressive phenotype with onset even in adulthood was described in 1976 [11], and this has been referred to as adrenomyeloneuropathy [12]. Despite the slower course, characterized mainly by progressive spastic paraparesis, this is fundamentally the same disorder and the two phenotypes have been observed in siblings [13]. The disease expresses in a portion of heterozygotes, in whom the picture is of adrenomyeloneuropathy.

The cholesterol esters found in the adrenal glands contain large amounts of very long-chain fatty acids (VLCFA) [8]. Moser and colleagues [14, 15] found that these elevated VLCFA could be demonstrated in blood and cultured fibroblasts, and this has become the method of choice for diagnosis. They can be demonstrated by gas chromatography and gas chromatography-mass spectrometry (GCMS). The oxidation of VLCFA takes place

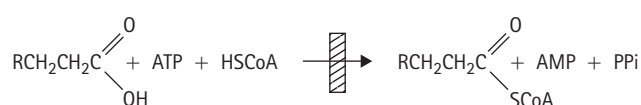


Figure 62.1 Very long-chain acylCoA synthetase (VLCAS), the activity is defective in adrenoleukodystrophy.

in peroxisomes. The enzyme that catalyzes the formation of the CoA esters of these very long-chain fatty acids is defective in this disorder (Figure 62.1) [16, 17]. However, the defective gene is that of a peroxisomal membrane transporter protein [18]. The gene was mapped to Xq28 [19]. It was isolated and found to be a member of the ABC transporter family [18, 20]. Of more than 200 mutations identified, approximately 50 percent were missense and 24 percent frame shifts; large deletions, insertions, and splicing defects are uncommon [21–23]. The gene is now referred to as *ABCD1* and its product as ALDP.

CLINICAL ABNORMALITIES

The presenting findings in adrenoleukodystrophy are often behavioral (Figures 62.2 and 62.3) [2, 9, 24, 25]. By four to eight years of age, some patients, who have passed early milestones normally, begin to be hyperactive and withdrawn. Others may be aggressive or belligerent, and this behavior may occur in bizarre outbursts. Initial referral is often to a psychiatrist or psychologist. Poor school performance may be another presenting problem, but often there is inattentiveness and poor concentration. Diagnosis of attention deficit disorder and treatment with stimulants, such as Ritalin, is a common history. There may be changes in or failing memory. Difficulty in communication or loss of acquired skills in speech is commonly encountered. In testing patients at risk, the earliest manifestations may be detected by neuropsychometric testing for abnormalities in visual or auditory processing, new learning or short-

term visual memory [26]. This is the classic or childhood cerebral onset phenotype that occurs in approximately 40 percent of patients. In a small number of these patients, onset is in adolescence, but most begin by three to ten years [2, 8, 24, 25].

Visual disturbances are common in these patients and may occur early. Homonymous hemianopsia has been observed in at least two patients [2], and there may be a striking loss in visual recognition of objects. There may be a transient horizontal nystagmus in the early stages of visual loss. There may be strabismus or double vision. Ultimately, virtually all patients have a loss of vision as a prominent feature. Optic atrophy is usually a late finding, but rarely it may be seen early. The pupillary response to light remains intact until late in the illness. Hearing loss is also characteristic and may occasionally be seen as an early finding. Difficulty in understanding speech in a noisy room or over the telephone may be an early sign of impaired auditory discrimination.

Abnormalities in gait may be seen early. Characteristically, the gait is stiff-legged and unsteady. Deep tendon reflexes may be increased. Asymmetry of relatively early findings may confuse the diagnosis. A number of patients have had hemiparesis, but eventually they all develop spastic quadriparesis. Astereognosis, graphesthesia, and apraxia may be seen early. Dysarthria and dysphagia occur regularly. Once they appear, neurologic manifestations are rapidly progressive, usually over months or years, to a decorticate state in which the patient is blind and deaf. Seizures are relatively common late in the disease and may be focal or generalized. In some

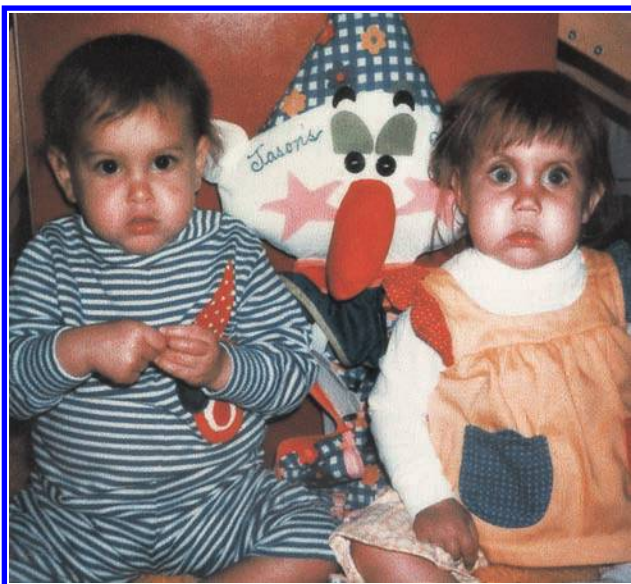


Figure 62.2 Two children with adrenoleukodystrophy. The diagnosis was made at six and eight years of age, respectively. Onset was with attention deficit and hyperactivity followed by loss of verbal skills. Hyperpigmentation was particularly prominent in inguinal areas.



Figure 62.3 Eight-year-old with adrenoleukodystrophy. The blank open-mouthed expression was characteristic. By nine years of age, he had spasticity and blind and had multiple contractures.

patients, they may occur early, even as the first neurologic feature. In a series of 167 patients, the duration of disease from the onset of neurological manifestations until death was 1.9 ± 2 ; range was 0.5–10.5 years [25].

The brown pigmentation may be evident at the onset of symptoms, but it is usually found later. It occurs particularly in areas not exposed to the sun, such as skin folds, inguinal areas, areolas, and the buccal mucosa. Clinical signs of adrenal insufficiency are seen in some patients and have antedated the neurologic manifestations in some patients [2]. For this reason, it is worth a specific assessment for adrenoleukodystrophy in any boy who develops adrenal insufficiency during childhood. The onset of adrenal failure is usually insidious, with fatigue and intermittent vomiting. Two patients have been reported who had arterial hypotension, but it is easy to see how the other manifestations of adrenal failure could be missed in a patient with advanced neurologic disease. The most useful test of adrenal function is to assess the response to adrenocorticotropin (ACTH) [25, 26]. Levels of cortisol in plasma or urinary 17-hydroxy steroids may be low, but more important is a failure to respond to ACTH. Testing with metyrapone early in the illness, when the ACTH stimulation test may be normal, reveals normal pituitary function. Concentrations of electrolytes in serum have regularly been normal. Plasma levels of ACTH may be increased.

Among variant presentations, patients with adrenomyeloneuropathy were so characterized because of prominent spinal cord involvement [11, 12]. Initial symptoms of stiffness or clumsiness in the legs progress to spastic paraplegia. Generalized weakness, loss of weight, hyperpigmentation, and attacks of vomiting are signs of adrenal insufficiency. The neurologic disease often progresses slowly over five to 15 years. The patient becomes wheelchair-bound and may develop problems with urination. Somatosensory and brainstem auditory evoked responses are abnormal. Cognitive function is abnormal in about half of the patients. Vibration sense in the lower extremities may be impaired and so may nerve conduction velocity. Impotence is common [27]. Gonadal insufficiency is ultimately common [28].

Symptoms in the female heterozygote may resemble those of adrenomyeloneuropathy. In a few, there is severe disability with paraparesis [29, 30]. These women may be thought to have multiple sclerosis, if there is no family history of involved males [31]. One patient had intermittent paresthesia from the age of 40 years [31]. Some asymptomatic patients have had hyperreflexia and impaired vibration sensation in the legs. Some have been diagnosed, only after they had an affected son [31]. Adrenal function is normal in most heterozygotes. Some have had dementia. Others have had an adolescent onset of the kind of progressive cerebral disease seen in the male, even with adrenal insufficiency [30, 32].

Rarely, males have had an adult onset of cerebral disease without cord involvement [29]. Some have been thought

to have schizophrenia, or Kluver-Bucy syndrome [33]. Psychotic symptoms in a patient with Addison disease should trigger this diagnosis, but adrenal function may be normal.

A small number of male patients, mostly from Japan, has presented with a picture of olivopontocerebellar atrophy [34–37]. Most were adults. A five-year-old Japanese child presented with cerebellar ataxia [38]. Imaging revealed cerebellar and pontine atrophy. The disease was progressive.

At the other end of the spectrum, three infants have been reported [39], whose phenotype was that of a peroxisomal disorder, such as neonatal ALD (Chapter 63). They had profound hypotonia, failure to thrive, and cholestatic hepatic disease. Two had seizures and the third episodic opisthotonus. In a single autopsy, the adrenals were small and fibrotic.

Some patients with this disease have had pure Addison disease without neurologic findings [39, 40]. In areas in which adrenal tuberculosis is rare, this disorder may represent a significant proportion of patients with Addison disease. Some patients, found by testing relatives of known patients, have been asymptomatic for long periods of time, but it is expected that sooner or later they will all develop neurologic abnormalities. Some have developed prominent cerebellar signs or a picture of olivopontocerebellar degeneration [35–37, 41].

Neuroimaging by computed tomography (CT) or

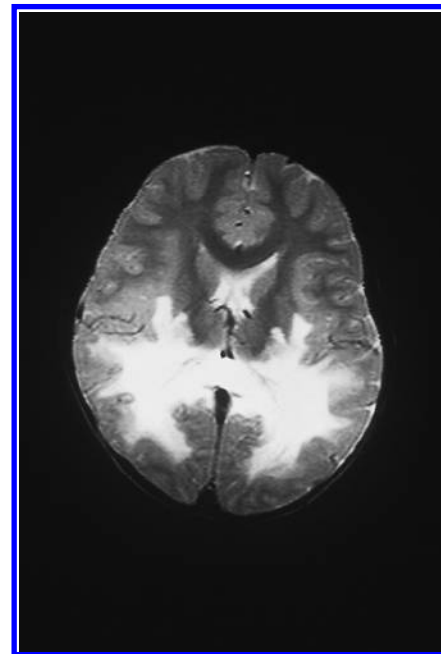


Figure 62.4 Magnetic resonance image of the brain of GQ, a seven-year-old boy with adrenoleukodystrophy. He had experienced the sudden onset of seizures after a period of disturbed behavior. He had pyramidal tract signs and spatial agnosia. Imaging revealed the typical white matter disease.



Figure 62.5 Computed tomographic scan of the brain of HM, a boy with adrenoleukodystrophy. There was extensive leukodystrophy and calcification around the lucent area of demyelination.

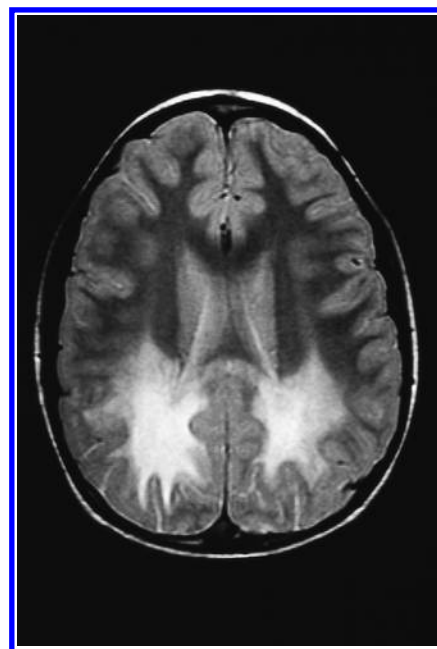


Figure 62.6 Magnetic resonance image of the brain of HM. The intense T_2 signal in the white matter was indicative of the leukodystrophy.

magnetic resonance imaging (MRI) [42–44] reveals evidence of leukodystrophy in the cerebral white matter (Figures 62.4, 62.5, and 62.6). Temporal and parieto-occipital involvement is seen most frequently, but there may be widespread involvement, including the cerebellar white matter and the corticospinal tracts. Some patients have had cerebral atrophy. There are widespread symmetric, confluent, low density lesions on CT or T_1 -weighted MRI or increased density on T_2 in the periventricular white matter of the parieto-occipital areas that enhance anteriorly in the CT on infusion of contrast material. Repeated scans over time show a caudal–rostral progression of the demyelination. The enhancement with contrast reflects breakdown of the blood–brain barrier, and this is seen also on brain scintiscan, which shows increased uptake in the involved areas. In some patients, the lesions found on imaging the white matter have been the first clue to the diagnosis. In a few patients, atypical unilateral lesions have, along with (in one patient) symptomatology of unilateral headache, visual loss, weakness, and hyperreflexia, led to a diagnosis of brain tumor [45]. Biopsy revealed leukodystrophy and led to the diagnosis. In asymptomatic patients at a mean age of 6.7 years, MRIs were normal and psychologic testing revealed normal cognitive function [46]. In 56 adult patients with white matter abnormalities, 42 had corticospinal disease, and 50 percent of these had progression of lesions over three to five years, but disease progression was slower than in affected children [47].

Brainstem auditory evoked responses in adrenoleukodystrophy may have abnormal [48] asymmetry in

that wave VI on one side may be absent. There may be progressive loss so that ultimately only a prolonged wave I is recordable bilaterally [49]. Electroencephalograms (EEG) are usually abnormal, most commonly showing diffuse slow activity or large-amplitude slow waves over the posterior regions [50].

Pathological examination of the nervous system reveals extensive diffuse demyelination in the cerebral white matter, most prominent in the occipital and posterior parietal areas and spreading in a caudal–rostral direction [2, 51, 52]. There is secondary loss of axons and gliosis. Late findings may be cavitation or calcification. In addition, there are diffuse perivascular infiltrations of lymphocytes. These inflammatory findings are not seen in adrenomyeloneuropathy, which is predominantly a distal axonopathy [52]. In the adrenal gland, there are ballooned cortical cells, which have characteristic striations [7].

The nature of the disorder was originally clarified by the finding of characteristic cytoplasmic inclusions in large glial cells or macrophages of the central nervous system and in adrenal cortical cells [51, 53]. The inclusions in the central nervous system stain positive with periodic acid-Schiff (PAS) and Oil Red O stains. Sudanophilia is common, but may be absent. The electron microscope reveals the pathognomonic ultrastructure of curvilinear spicules with central lucent spaces (Figures 62.7 and 62.8) [51, 53]. Similar inclusions have been seen in Schwann cells [54], making the diagnosis possible by sural nerve biopsy, but this has more often been negative [2]. Testicular tissue may show identical ultrastructural lamellar lesions

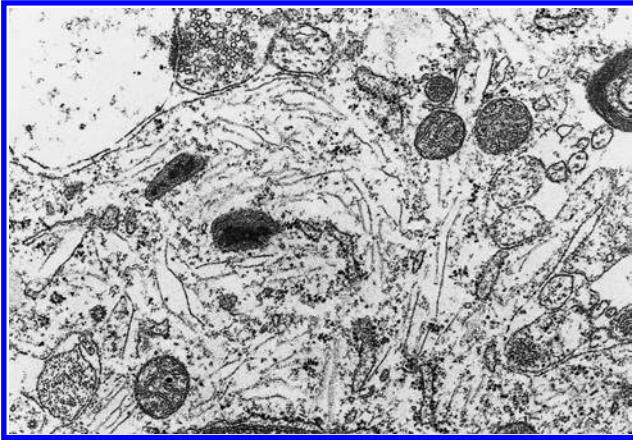


Figure 62.7 Electron microscopic section of the brain, illustrating the characteristic fine thin spicules. These cytoplasmic inclusions may be straight or curved and contain a central electrolucent space bound by a thin electrodense membrane. (Illustration kindly provided by Dr Henry Powell of the University of California, San Diego.)

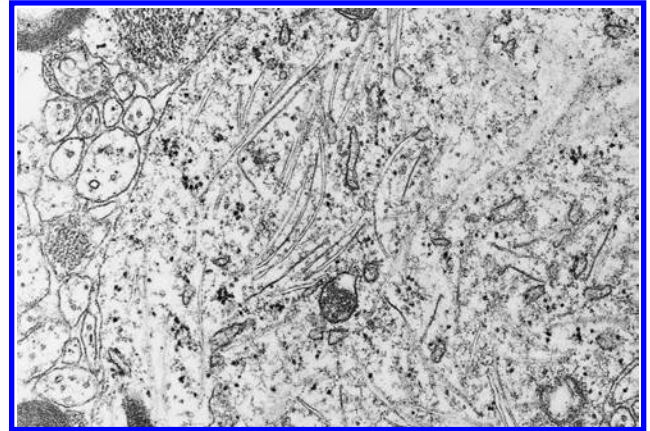


Figure 62.8 Cytoplasm of this brain cell illustrates the characteristic scimitar-shaped inclusions. (Illustration kindly provided by Dr Henry Powell of the University of California, San Diego.)

in Leyden cells [55]. Characteristic ultrastructural lesions have been seen in Schwann cells obtained by conjunctival biopsy, and vacuoles have been seen in eccrine glands of the skin [56]. Chemical methods have largely supplanted biopsy approaches to diagnosis.

GENETICS AND PATHOGENESIS

Adrenoleukodystrophy is an X-linked disorder that is not fully recessive in its expression, as there may be clinical expression in female heterozygotes.

The specific biochemical abnormality in adrenoleukodystrophy is the accumulation of very long-chain unbranched fatty acids, which are saturated or mono-unsaturated. The carbon lengths of these compounds are 24 to 30. They are found normally among the fatty acids of the cholesterol esters and gangliosides of the cerebral white matter and the adrenal cortex, and C26:0 makes up as much as 5 percent of the total fatty acids of cerebroside and sulfatides of the normal brain [8, 57–59]. Similarly, the VLCFA that accumulate in adrenoleukodystrophy are predominantly those with a chain length of 26. They are largely hexacosanoic acid (C26:0) (cerotic acid). Accumulation of these VLCFA has been demonstrated in cultured fibroblasts [14, 59, 60] and muscle cells [61]. In cultured fibroblasts, the ratio of C26 to C22 fatty acids has been useful in diagnosis, as well as the level of C26:0 [14]. The ratio was 0.76 in six patients with clinically typical disease and 0.78 in five patients with autopsy-proven disease, while in controls it was 0.06. The concentrations of these same very long-chain saturated fatty acids in plasma are also increased [62], and this is the most convenient method for definitive diagnosis. The levels of C24

(lignoceric acid), C25, and C26 are significantly elevated, while those of C20 and C22 are normal. The C26:C22 ratios of hemizygotes are approximately five times those of controls. In general, the plasma assay is sufficient for diagnosis. In instances in which the data are equivocal, the fibroblast assay is employed. The accumulation of VLCFA in patients with adrenomyeloneuropathy is no different than in patients with classic adrenoleukodystrophy [14].

The accumulation of VLCFA in adrenoleukodystrophy is a consequence of abnormality in the oxidation of these VLCFA, which takes place in peroxisomes [63, 64]. Studies of oxidation to $^{14}\text{CO}_2$ of ^{14}C -labeled fatty acids in fibroblasts revealed impaired production of CO_2 in adrenoleukodystrophy [65]. Oxidation of hexacosanoic (C26:0) acid was 14 percent of control, and that of lignoceric (tetracosanoic) (C24:0) was 8 percent of control.

Heterozygotes can be detected by assay of the VLCFA of plasma or cultured fibroblasts [65]. Levels in both plasma and fibroblasts, especially using the ratios of C26:C22 or C24:C22, were intermediate between patients and controls, and significantly different from the latter, but there was a small amount of overlap. By assaying both fibroblasts and plasma, over 90 percent of obligate heterozygotes can be identified [65]. Cloning of fibroblasts from heterozygotes yielded two populations of cells, one normal and the other identical to patients with adrenoleukodystrophy in its C26 fatty acid content [66]. These experiments proved that the gene is on the X chromosome and that it is subject to inactivation. Studies of women doubly heterozygous for glucose-6-phosphate dehydrogenase (G-6-PD) revealed close linkage between the two loci [66].

The gene for G-6-PD has been mapped to Xq28 [67], localizing the gene for adrenoleukodystrophy to this locus of the X chromosome. This has led to the exploration of

DNA probes, and DXS52 was found to be closely linked to adrenoleukodystrophy [68]. The only instance so far tested that looked like recombination turned out to be gonadal mosaicism in a grandmother [69]. The linkage can be used in heterozygote detection [70] and effectively resolves any situations in which the assay of VLCFA is not clear. Mutation analysis is the most reliable method for detection of heterozygosity [70].

The linkage to DXS52 has also been used successfully in prenatal diagnosis [71, 72]. The assay for VLCFA is usually employed as the procedure of choice for prenatal identification of the affected male fetus [73]. Cultured amniocytes and chorionic villus material have been used, but normal VLCFA have been found in cultured chorionic villus cells in pregnancies where the fetus turned out to be affected [74, 75]. Oxidation of C26:0 fatty acid in cultured chorionic villus material has been employed for prenatal diagnosis [76]. In terminated pregnancies, fetal ultrastructural as well as chemical abnormalities were clearly present. When the mutation is known, it provides a definitive method of prenatal diagnosis [77, 78].

Defective oxidation of VLCFA in this disease is a consequence of failure to form the coenzyme A esters. The synthetase enzyme (Figure 62.1) whose activity is defective has been localized to the peroxisome [79–81].

The gene for adrenoleukodystrophy was found by positional cloning within the Xq28 region [18]. It spans approximately 20 kb in ten exons. The deduced amino acid sequence placed it among the ATP-binding cassette superfamily of transmembrane transporter proteins [82]. The structure has extensive similarities to the 70 kDa peroxisomal membrane protein, PXMP 1 [18]. A few deletions in the gene were identified in patients by Southern blot analysis [18]. A majority of the mutations are missense, and a majority of all mutations are unique to the family in which they are found [23]. Mutations have clustered in the membrane spanning region and in the nucleotide binding region, and there is a hot spot in exon 5. Correlation between genotype and phenotype has not been possible. Polymorphism has also been identified (N13T) which caused amino acid change, but does not affect function of the ALDP [21]. In the three patients with neonatal presentations suggestive of peroxisomal disease [39], there was no immunochemically detectable ALDP, and large deletions were found in the *ABCD1* promoter region and the adjacent DXS1357E gene. Deletions in this latter gene cause creatine deficiency in brain as a result of deficiency in the X-linked creatine transporter gene (*SLC6A8*) [83]. Among 112 patients with adrenomyeloneuropathy, mutations were found in all of the probands [84]. They were scattered over the gene and did not correlate with phenotype. About 50 percent were missense mutations, of which 64 percent were at CpG dinucleotides. In a more recent review of mutations, the majority were point mutations [85]. Among the heterozygotes, the only reliable method of detection was mutation analysis; tests of VLCFA in plasma were

unreliable. In a three-year-old Japanese boy, a deletion of exons 3–10 of the *ABCD1* gene was fused to a neighboring gene, *PLNKB3* [86].

It is not clear how the defect in the gene affects VLCFA synthetase activity, and ALDP does not function as a synthetase. Nevertheless, it appears likely that the disease in the central nervous system and in the adrenal results from the accumulation of VLCFA [87, 88].

In studies of expression of the gene in tissues of patients, correlations with phenotype were elusive [89]. Accumulation of saturated VLCFA in white matter did correlate with phenotype.

Cloning of individual fibroblasts of heterozygotes demonstrated two populations of cells consistent with the Lyon hypothesis [90]. The content of C26 fatty acids was employed as the marker. There were more mutant clones than wild type, consistent with the fact that heterozygotes express disease phenotypes and a selective advantage of mutant cells. This contrasts with Lesch-Nyhan disease in which there is selection for wild type in females.

TREATMENT

The course of adrenoleukodystrophy has usually been relentless, and no therapeutic measures appear to be effective. Symptomatic therapy is important [91], and support groups may be helpful to families. Physiologic amounts of adrenal steroid replacement therapy are effective in the management of the adrenal disease.

Bone marrow transplantation has been carried out without improvement in neurologic status, but encouraging results have been obtained in patients treated early in their courses [92–96]. It is reported that some patients have become clinically stable after transplantation, and some have even improved. In patients studied with proton magnetic resonance spectroscopy, transplanted patients fell midway between controls and untreated patients in the ratios of N-acetylaspartate (NAA) to creatinine and NAA to choline [97]. Criteria for transplantation remain unclear, but increasingly patients have transplanted for worsening MRI before clinical regression.

Reduction in NAA and increase in choline on the magnetic resonance spectroscopy have been used as criteria for transplantation [94]. There is nevertheless increasing evidence [95, 96] that best results have come from early transplantation.

Gene therapy has been reported in two patients [98]. Autologous cells removed from the patients were transfected with a virus vector containing wild-type *ABCD1*. Over three months, there was polyclonal restitution in hematopoietic cells and T lymphocytes expressing the ALD protein and a cessation of cerebral demyelination.

Studies using deuterium-labeled hexacosanoic acid indicated that a substantial amount of the C26 fatty acids in the brain is of dietary origin [99]. Although there is also evidence that fibroblasts of patients are able, unlike

controls, to synthesize C26 fatty acid from stearic acid [100], these observations have raised the possibility of dietary therapy. Restriction of the intake of very long-chain fatty acids has been undertaken in this disease without effect on levels of VLCFA or clinical course. The observation [101] that the addition of mono-unsaturated fatty acids, such as oleic acid to cultured fibroblasts of patients, leads to reduction in accumulation of VLCFA, led to the use of glyceryltriolate in therapy. Glyceryltriolate was even more effective *in vitro* [102], and this has led to the development of Lorenzo's oil, a 4:1 mixture of triolate and trierucate oils, named after the patient whose parents popularized it, as shown in the film, 'Lorenzo's Oil'. Treatment does bring plasma levels of C26:0 to normal, but it is clear that the neurologic progression of the disease is not halted. Double blind placebo-controlled studies have not been done, but Lorenzo's oil does not appear to be useful in patients who have demonstrated neurological regression [103, 104]. It may be worth exploring in patients with adrenomyeloneuropathy, but the evidence is against it [105]. Many patients develop thrombocytopenia [106] and so platelet counts must be monitored. Levels of essential fatty acids should be monitored to prevent deficiency. In Moser's 2005 publication [107], 89 presymptomatic boys identified were treated with Lorenzo's oil and moderate restriction of the intake of fat. In a short follow up (6.9 ± 2.7 years), 29 percent developed abnormalities on MRI and 11 percent neurologic abnormalities. He recommended oil therapy in boys with normal MRI.

By analogy with its beneficial effect in sickle cell anemia, where they increase fetal hemoglobin, butyrate and 4-phenylbutyrate have been explored in adrenoleukodystrophy. Cultured cells from patients were found to have improvement in the oxidation of VLCFA, and amounts of stored VLCFA in the brain of a mouse model were decreased by exposure to phenylbutyrate [108]. Preliminary studies in man are said to be underway.

REFERENCES

1. Moser HW, Moser AB, Kawamura N *et al*. Adrenoleukodystrophy: studies of the phenotype genetics and biochemistry. *Johns Hopkins Med J* 1980; **147**: 217.
2. Schaumborg HH, Powers JM, Raine CS *et al*. Adrenoleukodystrophy. A clinical and pathological study of 17 cases. *Arch Neurol* 1975; **32**: 577.
3. Haberland W, Spieler F. Zur diffusen Hirn-Rueckenmarksklerose im Kindesalter. *Dtsch Z Nervenhe* 1910; **40**: 436.
4. Schilder P. Zur frage der encephalitis periaxialis diffusa (sogenannt diffuse sklerose). *Z Neuro Psych* 1913; **15**: 359.
5. Siemerling E, Creutzfeldt HG. Bronzkrankheit und sklerosierende encephalomyelitis. *Arch Psychiatr Nervenkr* 1923; **68**: 217.
6. Schaumborg HH, Richardson EP, Johnson PC *et al*. Schilder's disease: sex-linked transmission with specific adrenal changes. *Arch Neurol* 1972; **27**: 458.
7. Powers JM, Schaumborg HH. The adrenal cortex in adrenoleukodystrophy. *Arch Pathol* 1973; **96**: 305.
8. Igarashi M, Schaumborg HH, Powers JM *et al*. Fatty acid abnormality in adrenoleukodystrophy. *J Neurochem* 1976; **26**: 851.
9. Blaw ME. Melanodermic type leukodystrophy (adrenoleukodystrophy). In: Vinken PJ, Bruyn GW (eds). *Handbook of Clinical Neurology*, vol. 10. New York: American Elsevier, 1970: 128.
10. Fanconi VA, Prader A, Isler W *et al*. Morbus Addison mit hirschklerose im kindesalter D. Ein hereditares syndrom mit X-chromosomaler vererbung? *Helv Paediatr Acta* 1963; **18**: 480.
11. Budka H, Sluga E, Heiss WD. Spastic paraplegia associated with Addison's disease: adult variant of adrenoleukodystrophy. *J Neurol* 1976; **213**: 237.
12. Griffin JW, Goren E, Schaumborg H *et al*. Adrenomyeloneuropathy: a probable variant of adrenoleukodystrophy. *Neurology* 1977; **27**: 1107.
13. Davis LE, Snyder RD, Orth DN *et al*. Adrenoleukodystrophy and adrenoneuropathy associated with partial adrenal insufficiency in three generations of a kindred. *Am J Med* 1979; **66**: 342.
14. Moser HW, Moser AB, Kawamura N *et al*. Elevated C-26 fatty acid in cultured skin fibroblasts. *Ann Neurol* 1980; **7**: 542.
15. Moser HW, Moser AB, Frayer KK *et al*. Adrenoleukodystrophy: increased plasma content of saturated very-long-chain fatty acids. *Neurology* 1981; **31**: 1241.
16. Wanders RJA, van Roermund CWT, van Wijland MJA *et al*. Direct evidence that the deficient oxidation of very-long-chain fatty acids in X-linked adrenoleukodystrophy is due to an impaired ability of peroxisomes to activate very-long-chain fatty acids. *Biochem Biophys Res Commun* 1988; **153**: 618.
17. Lazo O, Contreras M, Hashmi M *et al*. Peroxisomal lignoceroyl-CoA ligase deficiency in childhood adrenoleukodystrophy and adrenomyeloneuropathy. *Proc Natl Acad Sci USA* 1988; **85**: 7647.
18. Mosser J, Douar AM, Sarde CO *et al*. Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. *Nature* 1993; **361**: 726.
19. Aubourg P, Sack GH, Meyers DA *et al*. Linkage of adrenoleukodystrophy to a polymorphic DNA probe. *Ann Neurol* 1987; **21**: 349.
20. Higgins CF. ABC transporters: from microorganisms to man. *Annu Rev Cell Biol* 1992; **8**: 67.
21. Dvorakova L, Storkanova G, Unterrainer G *et al*. Eight novel ABCD1 gene mutations and three polymorphisms in patients with X-linked adrenoleukodystrophy: the first polymorphism causing an amino acid exchange. *Hum Mutat* 2001; **18**: 52.
22. X-linked adrenoleukodystrophy database. Last accessed December. Available from: www.x-ald.nl.
23. Smith KD, Kemp S, Braiterman LT *et al*. X-linked adrenoleukodystrophy: genes mutations and phenotypes. *Neurochem Res* 1999; **24**: 521.
24. Aubourg P, Chaussain JL, Dulac O, Arthuis M. Adrenoleukodystrophy in childhood: a review of 20 cases. *Arch Fr Pediatr* 1982; **39**: 663.

25. Moser HW, Naidu S, Kumar AJ, Rosenbaum AE. The adrenoleukodystrophies. *CRC Crit Rev Neurobiol* 1987; **3**: 29.
26. Forsyth CC, Forbes M, Cummings JH. Adrenocortical atrophy and diffuse cerebral sclerosis. *Arch Dis Child* 1971; **46**: 273.
27. Powers JM, Schaumburg HH. A fatal cause of sexual inadequacy in men: adrenoleukodystrophy. *J Urol* 1980; **124**: 583.
28. Assies J, van Geel B, Barth P. Signs of testicular insufficiency in adrenomyeloneuropathy and neurologically asymptomatic X-linked adrenoleukodystrophy; a retrospective study. *Int J Androl* 1997; **20**: 315.
29. Moser HW, Moser AB, Naidu S, Bergin A. Clinical aspects of adrenoleukodystrophy and adrenomyeloneuropathy. *Dev Neurosci* 1991; **13**: 254.
30. Noetzel MJ, Landau WM, Moser HW. Adrenoleukodystrophy carrier state presenting as a chronic nonprogressive spinal cord disorder. *Arch Neurol* 1987; **44**: 566.
31. Dooley JM, Wright BA. Adrenoleukodystrophy mimicking multiple sclerosis. *Can J Neurol Sci* 1985; **12**: 73.
32. Heffungs W, Hameisier H, Ropers HH. Addison's disease and cerebral sclerosis in an apparently heterozygous girl: evidence of inactivation of the adrenoleukodystrophy locus. *Clin Genet* 1980; **18**: 184.
33. Powers JM, Schaumburg HH, Gaffney CL. Kluver-Bucy syndrome caused by adrenoleukodystrophy. *Neurology* 1980; **30**: 1131.
34. Tateish J, Sato Y, Suetsugu M, Takshiba T. Adrenoleukodystrophy with olivopontocerebellar atrophy-like lesions. *Clin Neuropathol* 1986; **5**: 34.
35. Marsden CD, Obeso JA, Lang AE. Adrenoleukomyeloneuropathy presenting as spinocerebellar degeneration. *Neurology* 1982; **32**: 1031.
36. Kuroda S, Kirano A, Yuasa S. Adrenoleukodystrophy: cerebello-brainstem dominant case. *Acta Neuropathol* 1983; **60**: 149.
37. Ohno T, Tsuchida H, Fukuhara N *et al.* Adrenoleukodystrophy: a clinical variant presenting as olivopontocerebellar atrophy. *J Neurol* 1984; **231**: 167.
38. Kurihara M, Kumagai K, Yagishita S *et al.* Adrenoleukomyeloneuropathy presenting as cerebellar ataxia in a young child: a probable variant of adrenoleukodystrophy. *Brain Dev* 1993; **15**: 377.
39. Corzo D, Gibson W, Johnson K *et al.* Contiguous deletion of the X-linked adrenoleukodystrophy gene (ABCD1) and DXS1357E: a novel neonatal phenotype similar to peroxisomal biogenesis disorders. *Am J Hum Genet* 2002; **70**: 1520.
40. Moser HW, Bergin A, Naidu S, Ladenson PW. Adrenoleukodystrophy: new aspects of adrenal cortical disease. *Endocrinol Metab Clin N Am* 1991; **20**: 297.
41. Takada K, Onoda J, Takahashi K *et al.* An adult case of adrenoleukodystrophy with features of olivo-ponto-cerebellar atrophy. *Jpn J Exp Med* 1987; **57**: 53.
42. Duda EE, Huttenlocher PR. Computed tomography in adrenoleukodystrophy: correlation of radiological and histological findings. *Radiology* 1976; **120**: 349.
43. Kumar AJ, Rosenbaum AE, Naidu S *et al.* Adrenoleukodystrophy: correlating MR imaging with CT. *Radiology* 1987; **165**: 496.
44. Aubourg P, Adamsbaum C, Lavallard-Rosseau MC *et al.* Brain MRI and electrophysiologic abnormalities in preclinical and clinical adrenomyeloneuropathy. *Neurology* 1992; **42**: 85.
45. Afifi AK, Menenez X, Reed LA, Bell WA. Atypical presentation of X-linked childhood adrenoleukodystrophy with an unusual magnetic resonance imaging pattern. *J Child Neurol* 1996; **11**: 497.
46. Cox CS, Dubey P, Raymond GV *et al.* Cognitive evaluation of neurologically asymptomatic boys with X-linked adrenoleukodystrophy. *Arch. Neurol* 2006; **63**: 69.
47. Eichler F, Mahnood A, Loes D *et al.* Magnetic resonance imaging detection of lesion progression in adult patients with X-linked adrenoleukodystrophy. *Arch Neurol* 2007; **64**: 659.
48. Black HA, Fariello RG, Chun RW. Brain stem auditory evoked response in adrenoleukodystrophy. *Ann Neurol* 1979; **6**: 269.
49. Kaga K, Tokoro Y, Tanaka Y, Ushijima H. The progress of adrenoleukodystrophy as revealed by auditory brainstem evoked responses and brainstem histology. *Arch Otorhinolaryngol* 1980; **228**: 17.
50. Mamoli B, Graf M, Toifi K. EEG pattern-evoked potentials and nerve conduction velocity in a family with adrenoleukodystrophy. *Electroencephalogr Clin Neurophysiol* 1979; **14**: 411.
51. Schaumburg HH, Powers JM, Suzuki K, Paine CS. Adrenoleukodystrophy (sex-linked Schilder's disease). Ultrastructural demonstration of specific cytoplasmic inclusions in the central nervous system. *Arch Neurol* 1974; **31**: 210.
52. Powers JM. Adrenoleukodystrophy (adreno-testiculo-leukomyelo-neuropathic-complex). *Clin Neuropathol* 1985; **4**: 181.
53. Powell H, Tindall R, Schultz P *et al.* Adrenoleukodystrophy: electron microscopic findings. *Arch Neurol* 1975; **32**: 250.
54. Powers JM, Schaumburg HH. Adrenoleuko-dystrophy: similar ultrastructural changes in adrenal cortical cells and Schwann cells. *Arch Neurol* 1974; **30**: 406.
55. Powers JM, Schaumburg HH. The testis in adrenoleukodystrophy. *Am J Pathol* 1981; **81**: 90.
56. Martin JJ, Ceuterick C, Martin L, Libert J. Skin and conjunctival biopsies in adrenoleukodystrophy. *Acta Neuropathol* 1977; **38**: 247.
57. Brown FR III, Chen WW, Kirschner DA *et al.* Myelin membrane from adrenoleukodystrophy brain white matter – isolation and physical/chemical properties. *J Neurochem* 1983; **41**: 341.
58. Igarashi M, Schaumburg HH, Powers J *et al.* Fatty acid abnormality in adrenoleukodystrophy. *J Neurochem* 1976; **26**: 851.
59. Kawamura N, Moser AB, Moser HW *et al.* High concentration of hexacosanoate in cultured skin fibroblast from adrenoleukodystrophy patients. *Biochem Biophys Res Commun* 1978; **82**: 114.
60. Tonshoff B, Lehnert W, Ropers H-H. Adrenoleukodystrophy: diagnosis and carrier. Detection by determination of long-chain fatty acids in cultured fibroblasts. *Clin Genet* 1982; **22**: 25.
61. Askanas V, McLaughlin JM, Engel WK, Adornato BT. Abnormalities in cultured muscle and peripheral nerve of a

- patient with adrenomyeloneuropathy. *N Engl J Med* 1979; **301**: 588.
62. Rezanker T. Very long chain fatty acids from the animal and plant kingdoms. *Prog Lipid Res* 1989; **28**: 147.
 63. Schutgens RBH, Heymans HSA, Wanders RJA *et al.* Peroxisomal disorders: a newly recognized group of genetic diseases. *Eur J Pediatr* 1986; **144**: 430.
 64. Singh I, Moser HW, Moser AB, Kishimoto Y. Adrenoleukodystrophy: impaired oxidations of long chain fatty acids in cultured skin fibroblasts and adrenal cortex. *Biochem Biophys Res Commun* 1981; **102**: 1223.
 65. Moser HW, Moser AB, Trojak JE, Supplee SW. Identification of female carriers of adrenoleukodystrophy. *J Pediatr* 1983; **103**: 54.
 66. Migeon BR, Moser HW, Moser AB *et al.* Adrenoleukodystrophy: evidence for X linkage inactivation and selection favoring the mutant allele in heterozygous cells. *Proc Natl Acad Sci USA* 1981; **78**: 5066.
 67. Pai GS, Sprenkle JA, Do TT *et al.* Localization of loci for hypoxanthine phosphoribosyltransferase and glucose-6-hypoxanthine dehydrogenase and biochemical evidence of nonrandom X-chromosome expression from studies of a human X-autosome translocation. *Proc Natl Acad Sci USA* 1980; **77**: 2810.
 68. Oberle I, Drayna D, Camerino G *et al.* The telomere of the human X-chromosome long arm: presence of a highly polymorphic DNA marker and analysis of recombination frequency. *Proc Natl Acad Sci USA* 1985; **82**: 2824.
 69. Graham CE, MacLeod PM, Lillcrap DP, Bridge PJ. Gonadal mosaicism in a family with adrenoleukodystrophy: molecular diagnosis of carrier status among daughters of a gonadal mosaic when direct detection of the mutation is not possible. *J Inherit Metab Dis* 1992; **15**: 68.
 70. Boehm CD, Cutting GR, Lachtermacher MB *et al.* Accurate DNA-based diagnostic and carrier testing for X-linked adrenoleukodystrophy. *Mol Genet Metab* 1999; **66**: 128.
 71. Boue J, Oberle I, Mandel JL *et al.* First trimester prenatal diagnosis of adrenoleukodystrophy by determination of very-long-chain fatty acid levels and by linkage analysis to a DNA probe. *Hum Genet* 1985; **69**: 272.
 72. Moser AB, Moser HW. The prenatal diagnosis of X-linked adrenoleukodystrophy. *Prenat Diagn* 1999; **19**: 46.
 73. Moser HW, Moser AB, Powers JM *et al.* The prenatal diagnosis of adrenoleukodystrophy. Demonstration of increased hexacosanoic acid levels in cultured amniocytes and fetal adrenal gland. *Pediatr Res* 1982; **16**: 172.
 74. Carey WF, Poulos A, Sharp P *et al.* Pitfalls in the prenatal diagnosis of peroxisomal beta oxidation defects by chorionic villus sampling. *Prenat Diagn* 1994; **14**: 813.
 75. Gray RGF, Green A, Cole T *et al.* A misdiagnosis of X-linked adrenoleukodystrophy in cultured chorionic villus cells by the measurement of very long chain fatty acids. *Prenat Diagn* 1995; **15**: 486.
 76. Wanders RJA, van Wijland MJA, van Roermund CWT *et al.* Prenatal diagnosis of Zellweger syndrome by measurement of very-long-chain fatty acid (C26: 0) beta-oxidation in cultured chorionic villus fibroblasts: implications for early diagnosis of other peroxisomal disorders. *Clin Chim Acta* 1987; **165**: 303.
 77. Maier EM, Roscher AA, Kammerer S *et al.* Prenatal diagnosis of X-linked adrenoleukodystrophy combining biochemical immunocytochemical and DNA analyses. *Prenat Diagn* 1999; **19**: 364.
 78. Imamura A, Suzuki Y, Song XQ *et al.* Prenatal diagnosis of adrenoleukodystrophy by means of mutation analysis. *Prenat Diagn* 1996; **16**: 259.
 79. Mannaerts GP, van Veldhoven P, Van Broekhoven A *et al.* Evidence that peroxisomal acyl-CoA synthetase is located at the cytoplasmic side of the peroxisomal membrane. *Biochem J* 1982; **204**: 17.
 80. Lageweg W, Tager JM, Wanders JA. Topography of very-long-chain fatty acid activating activity in peroxisomes from rat liver. *Biochem J* 1991; **276**: 53.
 81. Lazo O, Contreras M, Yoshida Y *et al.* Cellular oxidation of lignoceric acid is regulated by the subcellular localization of lignoceroyl-CoA ligases. *J Lipid Res* 1990; **31**: 583.
 82. Sarde C-O, Mosser J, Kioschis P *et al.* Genomic organization of the adrenoleukodystrophy gene. *Genomics* 1994; **23**: 13.
 83. Salomons GS, van Dooren SJ, Verhoeven NM *et al.* X-linked creatine-transporter gene (SLC6A8) defect: a new creatine-deficiency syndrome. *Am J Hum Genet* 2001; **68**: 1497.
 84. Kok F, Neumann S, Sarde CO *et al.* Mutational analysis of patients with X-linked adrenoleukodystrophy. *Hum Mutat* 1995; **6**: 104.
 85. Kemp S, Pujol A, Waterham HR *et al.* ABCD1 mutations and the X-linked adrenoleukodystrophy mutation database: role in diagnosis and clinical correlations. *Hum Mutat* 2001; **18**: 499.
 86. Matsumoto T, Miyake N, Watnabe Y *et al.* X-linked adrenoleukodystrophy with partial deletion of ALD due to fusion with the neighbour gene, PLXNB3. *Am J Med Genet* 2005; **138A**: 300.
 87. Theda C, Moser AB, Powers JM, Moser HW. Phospholipids in X-linked adrenoleukodystrophy white matter – fatty acid abnormalities before the onset of demyelination. *J Neurol Sci* 1992; **110**: 195.
 88. Reinecke CJ, Knoll DP, Pretorius PJ *et al.* The correlation between biochemical and histopathological findings in adrenoleukodystrophy. *J Neurol Sci* 1985; **70**: 21.
 89. Asheuer M, Bieche I, Laurendeau I *et al.* Decreased expression of ABCD4 and BG1 genes early in the pathogenesis of X-linked adrenoleukodystrophy. *Hum Molec Genet* 2005; **14**: 1293.
 90. Migeon BR, Moser HW, Moser AB *et al.* Adrenoleukodystrophy: evidence for X linkage inactivation, and selection favouring the mutant allele in heterozygous cells. *Proc Natl Acad Sci USA* 1981; **78**: 5066.
 91. Brown FR III, Stowens DW, Harris JC Jr, Moser HW. The leukodystrophies. In: Johnson RT (ed.). *Current Therapy in Neurologic Disease*. Philadelphia, PA: Marcel Dekker, 1985: 313.
 92. Aubourg P, Blanche S, Jambaque I *et al.* Reversal of early neurologic and neuroradiologic manifestations of X-linked adrenoleukodystrophy by bone marrow transplantation. *N Engl J Med* 1990; **322**: 1860.
 93. Shapiro E, Krivit W, Lockman L *et al.* Long-term effect of bone-marrow transplantation for childhood-onset cerebral X-linked adrenoleukodystrophy. *Lancet* 2000; **356**: 713.

94. Kruse BK, Barker PB, van Zijl PCM *et al*. Multislice proton magnetic resonance spectroscopic imaging in X-linked adrenoleukodystrophy. *Ann Neurol* 1994; **36**: 595.
95. Peters C, Charnas LR, Tan Y *et al*. Cerebral X-linked adrenoleukodystrophy: the international hematopoietic cell transplantation experience from 1982 to 1999. *Blood* 2004; **104**: 881.
96. Schonberger S, Roerig P, Schneider DT *et al*. Genotype and protein expression after bone marrow transplantation for adrenoleukodystrophy. *Arch Neurol* 2007; **64**: 652.
97. Rajanayagam V, Grad J, Krivit W *et al*. Proton MR spectroscopy of childhood adrenoleukodystrophy. *Am J Neuroradiol* 1996; **17**: 1013.
98. Cartier N, Hacein-Bey-Abina S, Bartholomae CC *et al*. Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. *Science* 2009; **326**: 818.
99. Kishimoto Y, Moser HW, Kawamura N *et al*. Adrenoleukodystrophy: evidence that abnormal very long-chain fatty acids of brain cholesterol esters are of exogenous origin. *Biochem Biophys Res Commun* 1980; **96**: 69.
100. Tsuji S, Sano T, Ariga T, Miyatake T. Increased synthesis of hexacosanoic acid (C26: 0) by cultured skin fibroblasts from patients with adrenoleukodystrophy (ALD) and adrenomyeloneuropathy (AMN). *J Biochem* 1981; **90**: 1233.
101. Rizzo WB, Watkins PA, Phillips MW *et al*. Adrenoleukodystrophy: oleic acid lowers fibroblast saturated C22:C26 fatty acids. *Neurology* 1986; **36**: 357.
102. Rizzo WB, Leshner RT, Odone A *et al*. Dietary erucic acid therapy for X-linked adrenoleukodystrophy. *Neurology* 1989; **39**: 1415.
103. van Geel BM, Assies J, Haverkort EB *et al*. Progression of abnormalities in adrenomyeloneuropathy and neurologically asymptomatic X-linked adrenoleukodystrophy despite treatment with 'Lorenzo's oil'. *J Neurol Neurosurg Psychiatry* 1999; **67**: 290.
104. Moser HW. Lorenzo oil therapy for adrenoleukodystrophy: a prematurely amplified hope. *Ann Neurol* 1993; **34**: 121.
105. Aubourg P, Adamsbaum C, Lavallard-Rousseau M-C *et al*. A two-year trial of oleic and erucic acids ('Lorenzo's oil') as treatment for adrenomyeloneuropathy. *N Engl J Med* 1993; **329**: 745.
106. Zinkham WH, Kickler T, Borel J, Moser HW. Lorenzo's oil and thrombocytopenia in patients with adrenoleukodystrophy. *N Engl J Med* 1993; **328**: 1126.
107. Moser HW, Raymond GV, Lu S-E *et al*. Follow-up of 89 asymptomatic patients with adrenoleukodystrophy treated with Lorenzo's oil. *Arch Neurol* 2005; **62**: 1073.
108. Lu J-F, Lawler AM, Watkins PA *et al*. A mouse model for X-linked adrenoleukodystrophy. *Proc Natl Acad Sci USA* 1997; **94**: 9366.

Introduction	469	Treatment	476
Clinical abnormalities	470	References	476
Genetics and pathogenesis	474		

Profound hypotonia, seizure disorder, hepatic fibrosis, atrophic adrenals, accumulation of very long-chain saturated fatty acids, pipecolic aciduria, and defective biogenesis of peroxisomes as a result of failure to import peroxisomal proteins.

Neonatal adrenoleukodystrophy was first described in 1978 by Ulrich and colleagues [1]. A relatively small number of patients has since been recognized [2–12]. Accumulation of very long-chain fatty acids (VLCFA) in this condition is indicative of multiple defective peroxisomal functions. This disorder, infantile Refsum disease, hyperpipecolic aciduria, and Zellweger syndrome fall into the same group of disorders of peroxisomal biogenesis, but at least 12 complementation groups have been identified, indicating fundamental defects in a number of steps in peroxisomal biogenesis [13–15]. Among them, Zellweger is the most severe, but neonatal adrenoleukodystrophy is also a very

severe disease. No correlation between complementation group and phenotype has emerged. Zellweger syndrome is found in at least ten complementation groups and neonatal adrenoleukodystrophy in at least six of the same groups. Among patients with Zellweger syndrome, one has been shown to have a defect in peroxisomal assembly factor 1 (PAF1), a 35-kDa membrane protein involved in the assembly of peroxisomes [16]. Two patients were found to have mutated alleles in the 70-kDa peroxisomal membrane protein (PMP70) [17], which is a member of the multiple drug resistance-related adenosine triphosphate (ATP)-binding cassette transporter superfamily. Patients with defective biogenesis of peroxisomes have abnormality in virtually every peroxisomal function, notably the

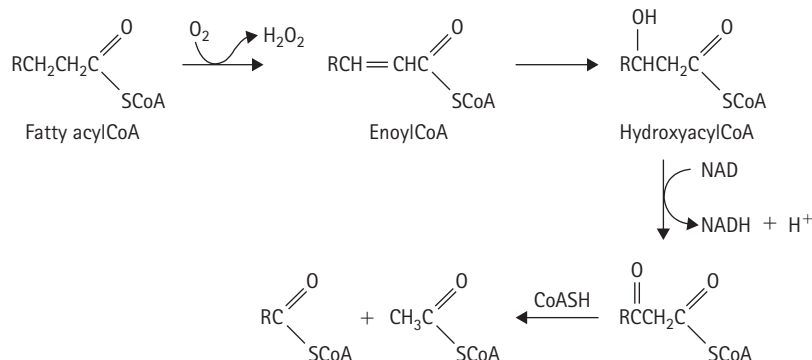


Figure 63.1 The pathway of carnitine-independent oxidation in peroxisomes. The NAD-dependent step and the one preceding it are analogous to mitochondrial enzymes enoylCoA hydratase and 3-hydroxyacylCoA dehydrogenase and they are catalyzed by the bifunctional enzyme. This pathway catalyzes the metabolism of long- and medium-chain fatty acids, as well as the very long-chain fatty acids. Short-chain acylCoAs and acetylCoA can then be transferred into carnitine esters via peroxisomal carnitine transferases.

peroxisomal β -oxidation of fatty acids (Figure 63.1).

Another peroxisomal assembly protein, PXR1, is the site of the defect in some patients with neonatal adrenoleukodystrophy. Two mutations have been identified: a nonsense and a missense mutation [18]. Genes for peroxisomal biogenesis in yeast have been extensively studied; they are referred to as PEX genes, and this usage has been adopted for the human orthologs [19]. Proteins that mediate the import of peroxisomal matrix proteins are called peroxins, and they are encoded by PEX genes. Members of the various complementation groups have mutations in what are now known as the human PEX genes. Thus, the mutations in the patient with neonatal adrenoleukodystrophy, referred to initially as PXR1 are in the PEX 5 gene on chromosome 12p13. PEX1, the first PEX gene to be identified in yeast [20], situated on human chromosome 7q21-22 is the most common cause of neonatal adrenoleukodystrophy and Zellweger syndrome [21].

CLINICAL ABNORMALITIES

The clinical picture of neonatal adrenoleukodystrophy is dominated by extreme hypotonia and a severe convulsive disorder (Figures 63.2, 63.3, 63.4, and 63.5) [1, 3, 9]. The hypotonia may be evident on the initial neonatal examination and is severe enough to suggest a diagnosis of myopathy [9]. Cerebral manifestations are profound. Most of these infants show little evidence of psychomotor development. Sucking reflex is poor. They feed poorly



Figure 63.2 AA: At 3½ months of age, illustrating the hypotonia and the ptosis on the left. She had a peroxisome biogenesis disorder resulting from mutation T1467G in the PXR gene [18].

and may fail to thrive unless tube-fed. There are few or no spontaneous movements. The grasp and Moro responses are poor or absent. Tonic neck, stepping, and placing reactions are absent. Deep tendon reflexes are usually diminished. Two patients were reported to be macrocephalic [3]. Ocular abnormalities reported include nystagmus, optic atrophy, and pigmentary degeneration of the retina [1, 7, 22, 23].

Seizures usually begin within the first days of life and continue as a major problem. They tend to be refractory to anticonvulsant therapy. Seizures may be myoclonic, as well as grand mal. Shivering or trembling may be stimulated by light touch and may be reminiscent of autumn leaves. Electroencephalograms (EEG) are abnormal, usually showing multifocal spike discharges [1, 3]. In one patient, the pattern changed at one month to that of hypsarrhythmia [1]. Decreased nerve conduction has been reported [3]. The computed tomography (CT) scan may be normal, or show a mild decrease in white matter, or there may be many patchy lucencies on CT scan or magnetic resonance imaging (MRI) [3]. There may be enlargement of the ventricular system. Cerebrospinal fluid protein may be elevated [1, 3].

Hepatomegaly may be progressive [3]. Levels of transaminase activity in the blood may be elevated [3]. With time, there is little evidence of developmental



Figure 63.3 Baby girl M: A neonate with neonatal adrenoleukodystrophy who presented with severe neonatal seizures. She was extremely hypotonic. She trembled at slight touch. There was mild hepatomegaly and absent neonatal reflexes. Magnetic resonance imaging was consistent with abnormal myelination. Analysis of very long-chain fatty acids (VLCFA) revealed a C26 of 3.25 $\mu\text{g}/\text{mL}$ and a C26/C22 ratio of 1.05. She died at three or four months of age at home.



Figure 63.4 AH: A six-month-old boy who had severe early infantile myoclonic seizures. Very long-chain fatty acids (VLCFA) were elevated. He died at one year of age.



Figure 63.6 HS: A one-month-old girl with Zellweger syndrome, had the typical facial appearance along with severe hypotonia, absent development, and hepatomegaly. Very long-chain fatty acids (VLCFA) were elevated. She died at four months of age.



Figure 63.5 AH: The brother of the patient in Figure 63.4 at four months. He had uncontrollable early infantile seizures and elevated very long-chain fatty acids (VLCFA). He died at ten months of age.



Figure 63.7 The ear of HS.

progress. One patient could smile and roll from supine position at between seven and nine months, but lost these functions shortly thereafter. Another developed some lateral head movement and a smile, which she lost after 12 months [3]. One patient had a cataract [3]. Most patients have died before the second birthday [1, 3, 9]. Mean age of death of the patients was 15 months, while in classical Zellweger syndrome it was 5.7 months. In neonatal adrenoleukodystrophy, death has occurred as early as

four months. A small number of patients has survived to teenage, albeit severely handicapped and dysmorphic [24, 25]. Impaired hearing and retinopathy have suggested a diagnosis of Usher syndrome. The mental age of patients has seldom exceeded 12 months and some have regressed at three to five years.

Dysmorphic features may be like those of Zellweger syndrome, but may be absent [9, 10]. Renal cysts are not found, nor is chondrodysplasia punctata. The typical



Figure 63.8 MB: An infant with Zellweger syndrome whose clinical and chemical presentation was like that of HS, but who was alive at 18 months when the family was lost to follow up. The forehead was striking.



Figure 63.10 The ear of AM.



Figure 63.9 AM: A four-month-old infant with Zellweger syndrome and the typical facies. He had cataracts, hypotonia, and retinitis. The patellae were stippled. Very long-chain fatty acids (VLCFA) were elevated, and there was pipeocolic aciduria. He died at six months of age.

facial appearance of the Zellweger syndrome includes a prominent high forehead and flat occiput with large fontanels and wide cranial sutures, abnormal helices of the ears, a broad nasal bridge, epicanthal folds, and hypoplastic supraorbital ridges (Figures 63.6, 63.7, 63.8, 63.9, and 63.10) [26, 27]. In addition, there are hepatorenal abnormalities and stippled calcifications in the patellae. Nipples and external genitalia may be hypoplastic.

The advent of molecular understanding of the disorders of peroxisomal biogenesis may ultimately render the earlier distinct clinical phenotypes obsolete. It is clear that there is a spectrum from the very severe Zellweger phenotype to the severe neonatal adrenoleukodystrophy to the more indolent infantile Refsum disease, and that mutations in the same gene can produce any of these phenotypes. The infantile Refsum phenotype may include some dysmorphic features, such as epicanthal folds, a flat nasal bridge, and low set ears [28]. Hypotonia is impressive in all of these diseases [29]. Retinitis pigmentosa and sensorineural hearing loss are characteristic [30]. These patients learn to walk but with an ataxic gait and they are severely impaired [29].

The same phenotype can also be found in deficiencies of single peroxisomal enzymes (Figures 63.11, 63.12, and 63.13). The enzymes include acylCoA oxidase-1 [31, 32], which phenotype has been referred to as pseudoneonatal adrenoleukodystrophy, D-bifunctional protein [33, 34], and peroxisomal thiolase-1 [35], which phenotype was originally referred to as pseudo-Zellweger syndrome. In these disorders, VLCFA are elevated, but plasmalogen synthesis is normal. Among these disorders,



Figure 63.11 The tigroid retinitis pigmentosa of SM, a girl with peroxisomal bifunctional protein deficiency.

deficiency of the D-bifunctional enzyme is much more common. This protein has both enoylCoA hydratase and 3-hydroxyacylCoA dehydrogenase activity. Among these patients three subgroups have been identified [35, 36], one with deficient hydratase activity, one with deficient hydroxyacyl dehydrogenase activity, and one with absent protein and deficiency of both activities.

Patients with neonatal adrenoleukodystrophy usually have no clinical evidence of adrenal insufficiency. Electrolyte concentrations are normal. One patient had a low level of cortisol in the serum, but the cortisol response to adrenocorticotropin (ACTH) was normal. Most have had impaired cortisol responses to ACTH. One



Figure 63.12 SE: A neonate with a typical neonatal adrenoleukodystrophy phenotype with intractable seizures and essentially no muscle tone. Very long-chain fatty acids (VLCFA) were highly elevated, but plasmalogens were normal, indicating a single enzyme in peroxisomal fatty acid oxidation rather than a defect in peroxisomal biogenesis. A deficiency of the D-bifunctional protein was likely, but has not yet been tested. (Patient was kindly referred by Dr Keith Vaux.)

patient developed hypoglycemia in response to fasting [9]. Very small adrenals have been observed at autopsy [1, 3]. Histological examination of adrenals has revealed

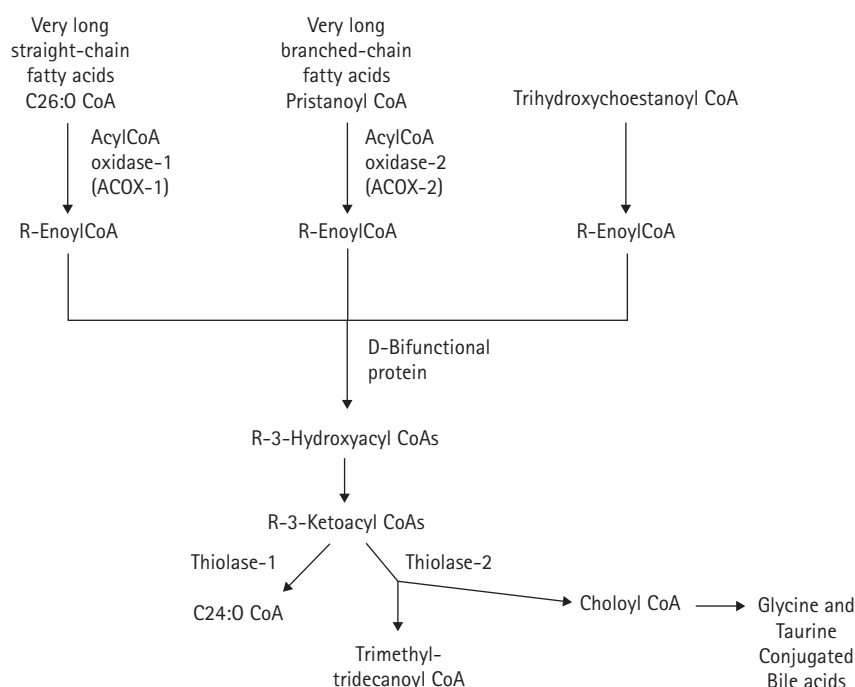


Figure 63.13 Peroxisomal β -oxidation of very long-chain fatty acids.

extensive cortical atrophy with nodules of ballooned cells that stained for lipid with Oil Red O. Electron microscopy showed lamellar, needle-like lipid inclusions. It was this similarity to X-linked adrenoleukodystrophy that led to the naming of this disease. This adrenal pathology is also seen in Zellweger syndrome [37]. ACTH was demonstrated immunohistochemically in the pituitary, indicating the adrenal changes to be primary [1].

The neuropathology is characterized in some patients by polymicrogyria, as well as patchy demyelination throughout the cerebral white matter [1]. Some have had only mild abnormalities in neuronal migration and heterotopias [11]; in others the cortex and neurons appeared normal [6, 38]. The olivary nuclei were normal in all. Cytoplasmic inclusions were seen, as in the adrenal, and were like those seen in X-linked adrenoleukodystrophy, as was perivascular accumulation of the lymphocytes. Demyelination of a widespread sudanophilic leukodystrophy tends to be more extensive than in X-linked adrenoleukodystrophy. It includes the cerebellum and brainstem. Periventricular rarefactions and microcalcifications have been observed [3]. Abnormalities of the gray matter include neuronal loss and inclusions in cortical neurons. Ocular histopathology includes ganglion cell loss and retinitis pigmentosa-like changes [22]. Retinitis pigmentosa may be seen in a variety of the peroxisomal disorders. [Figure 63.12](#) illustrates the retina of a patient with defective activity of the bifunctional enzyme protein, which is a single enzyme defect in the pathway of peroxisomal β -oxidation ([Figure 63.1](#)) rather than a defect in peroxisome assembly.

Extensive hepatic fibrosis was reported in two patients at autopsy [3] and periportal fibrosis was observed at biopsy at three months of age [9]. Periodic acid-Schiff (PAS)-positive macrophages have been reported in the liver [6], but not uniformly [3]. Hepatic peroxisomes may be absent or diminished in number [12, 39, 40].

Chemical analysis of the lipid of the brain revealed an increase in cholesterol esters and a diminution in constituents of myelin [1]. Hexacosanoic (C26:0) acid accounted for 25 percent of the total fatty acid [24, 41]. Examination of the very long-chain fatty acids of the plasma and cultured fibroblasts also reveals accumulation of VLCFA. Levels are similar to those found in X-linked adrenoleukodystrophy [4]. The mean C26:C22 ratio in fibroblasts in two patients [3] was 0.5, while that in adrenoleukodystrophy was 0.7. The value for controls was 0.03. The accumulation tends to be less than that seen in Zellweger syndrome. In another patient, the ratio was 1.8 [9]. The levels of C26:0 in postmortem liver and adrenal were higher than those reported in adrenoleukodystrophy [3]. Accumulation of very long-chain fatty acids has also been observed in retina [23]. Oxidation of lignoceric acid (C24:0) in cultured fibroblasts is impaired [3], and the level of activity is similar to that of cells derived from patients with adrenoleukodystrophy. Defective plasmalogen synthesis tends to be less than that of Zellweger syndrome [42].

GENETICS AND PATHOGENESIS

All of the disorders of peroxisomal biogenesis are transmitted in an autosomal recessive fashion. Patients of both sexes have been reported with identical phenotypes. In the Zellweger syndrome, consanguinity was observed in 17 of 78 patients [43]. The frequency of Zellweger syndrome was estimated to be one in 100,000 [44] and that of all disorders of peroxisome biogenesis to be one in 25,000 to 50,000 [27].

Testing of parents for levels of very long-chain fatty acids in plasma and in fibroblasts yielded normal levels [3]. This is in contrast to the findings in X-linked adrenoleukodystrophy. Normal levels of very long-chain fatty acids have also been found in plasma and fibroblasts of parents of patients with Zellweger syndrome [41].

Prenatal diagnosis has been accomplished in Zellweger syndrome [41], as it has been in X-linked adrenoleukodystrophy [45], by assay of cultured amniocytes, or chorionic villus samples, for very long-chain fatty acids and/or the activity of dihydroxyacetonephosphate acyltransferase [46–48], as well as by demonstration that catalase activity is present in the cytosol [49]. The same approaches should also be effective in neonatal adrenoleukodystrophy. False negatives have been observed in testing chorionic villus samples, so it has been recommended that tests be followed up by testing cultured chorionic villus cells, though this would not obviate the problem of overgrowth of maternal cells [45, 50]. In patients shown to have mutations in any of the PEX genes [16, 17, 51], these can be the basis of prenatal diagnosis and heterozygote detection.

The fundamental defect in the disorders of peroxisomal biogenesis is a failure in the process of protein import into the peroxisomal matrix. This may lead to an absence of demonstrable peroxisomes as reported in Zellweger syndrome [52]. Actually, fibroblasts of these patients have been shown to have peroxisomal ghosts, or empty peroxisomal structures containing membrane proteins, but no catalase or other matrix proteins [53–56]. In milder examples, including some patients with neonatal adrenoleukodystrophy, there may be small amounts of catalase within the ghosts [57].

Peroxisomal biogenesis requires the synthesis of proteins on cytosolic polyribosomes and post-translational import to pre-existing peroxisomes, which enlarge until they divide and form new peroxisomes. Matrix proteins include catalase, the bifunctional hydratase-dehydrogenase enzyme, the thiolase, and acylCoA oxidase [6]. Peroxisomal matrix proteins carrying either a carboxy terminal peroxisomal targeting sequence (PTS1) or a cleavable amino terminal sequence (PTS2) are translocated across the peroxisomal membrane [58–60]. A defect in a peroxin, caused by mutation in a PEX gene leads to failure of protein import via either the PTS1 or PTS2 import pathway and, as a consequence, to functional deficiency of the peroxisomes.

The PEX1 gene codes for a member of the AAA protein

family of ATPases, which interacts with another ATPase coded for by *PEX6*, and this interaction is required for matrix protein import [61, 62]. The cDNA codes for a hydrophilic protein of 1283 amino acids [62]. Defects in the *PEX1* gene account for over half of patients with defects of peroxisomal biogenesis [63–65]. A variety of mutations has been identified. Two mutations, G843D [21] and 2097insT [66], are common in the general population; one or the other allele accounts for a great majority of patients with defects in peroxisomal biogenesis [63, 64]. *PEX1* mutations lead to severe defects in matrix protein import and destabilization of *PEX5*, the receptor for the type 1 peroxisomal targeting signal [23]. Genotype tends to correlate with phenotype in the sense that missense mutations have been found in milder presentations and nonsense mutations, deletions, and insertions in severe disease [64]. Thus, the type of mutation can be helpful in prognosis. The G843D mutation not only leads to milder disease in the homozygote, but also appears to ameliorate the effects of genes that usually cause severe disease. It was found in homozygous fashion in at least one patient with neonatal adrenoleukodystrophy (ALD) [23] and several with infantile Refsum disease. The mutation was found on one of two alleles in patients with Zellweger syndrome, as well as these two diseases [62]. A frameshift mutation in exon 18 was relatively common in Australasian patients [63]. In an assembly of 168 patients, p.G843D and c.9097 insT accounted for more than 80 percent of abnormal alleles. Class I mutations led to residual protein concentrations and function, and milder disease, while class II led to no or almost no *PEX* protein and a severe phenotype [67]. Mutations causing premature termination were widely distributed through the gene, while missense mutations were concentrated in the essential AAA domains of the *PEX1* protein [68].

PEX2 deficiency is defective in patients in complementation group 10 [16]. The gene is on chromosome 8q21.1 [69]. It codes for a 35-kDa peroxisomal membrane protein that restores proper assembly in a CHO cell mutant that is defective in peroxisome assembly [70]. In the initial homozygous patient, a point mutation led to a premature termination of the protein, and addition of wild-type protein to cultured cells of the patient restored peroxisomal assembly. Other point mutations in this protein have been identified [71], a G-to-A 737 change that led to a cysteine-to-tyrosine change in the region of the carboxyl terminus resembling the zinc finger motif of DNA binding proteins, and a C-to-T 370 change which formed a stop codon.

The identification of the molecular defect in the *PEX5* gene, defective in complementation group 2 was the result of imaginative studies [18] on the patient [9] shown in Figure 63.2. She was found to have a T-to-G transversion of nucleotide 1467 which changed an asparagine to a lysine. The same mutation was found in another, unrelated Arabian patient with a neonatal adrenoleukodystrophy phenotype in complement group 2 [72]. Another member

of complement group 2 was found to be homozygous for a nonsense mutation T1168C that changed an arginine to a premature termination in *PXR1* [18]. *PEX1* codes for a receptor for proteins with PTS1 targeting signals, the targeting signal on the majority of matrix proteins. Fibroblasts of these patients were unable to import PTS1-containing proteins into peroxisomes. Solution of the crystal structure of the *PEX5* protein revealed major changes from an open structure to a closed circle when cargo is bound [73]. Mutations within the loop led to defective import of cargo into peroxisome.

The *PEX10* gene codes for a protein of 326 amino acids and a transmembrane pattern. Mutations such as nonsense, frameshift, or splice site that removed large segments of the coding region were found in Zellweger syndrome [74], while in a mildly affected patient with neonatal ALD, a missense mutation p.H290Q was in the C-terminal Zn binding domain. The loss of *PEX10* as seen in complementation group 7 affected predominantly the input of matrix protein.

Mutation in *PEX13* is responsible for patients in complementation group 13 [75]. The gene maps to chromosome 2p15. *PEX13* functions as a docking factor for the cytoplasmic PTS1 receptor [75]. Newly synthesized peroxisomal matrix proteins are distinguished by the presence of a PTS. Type 1 PTS has a C-terminal tripeptide ser-lys-lev, which is used for virtually all proteins destined for the peroxisomal lumen. In a patient with Zellweger syndrome, homozygosity for the nonsense mutation p.trp234ter led to loss of the transmembrane domain of the *PEX13* protein [75]. In a patient with neonatal ALD with a temperature-sensitive phenotype in fibroblast, there was homozygosity for a missense mutation p.I326T [76].

Mutations in other genes involved in the import of peroxisomal matrix proteins have been identified, in the *PEX6* [61], *PEX7* [77], and *PEX12* [78]. In addition, mutations have been observed in *PEX3* [79], *PEX16* [80], and *PEX19* [81], which code for proteins of peroxisomal membrane synthesis, all of them in patients with the Zellweger phenotype.

Mutations have been found in *PEX26*, the cause of peroxisome biogenesis complementation group 8 [82–84]. The gene was mapped to chromosome 22q11.2. It interacts directly with *PEX6*, and through it to *PEX1*. This relatively common group contains patients with all three phenotypes. Cells of these patients have defective import of catalase and PTS2 proteins, such as 3-ketoacylCoA thiolase, but not PTS1 proteins, and the import defect is temperature sensitive [82]. The temperature-sensitive variants tend to cause less severe disease, infantile ALD, or Refsum, than the nonsensitive variants. Among 18 probands in complementation group 8, p.R98W accounted for 14 (39 percent) of variant genes, and they hypothesized a founder effect [85].

In the presence of defective processing of peroxisomal matrix proteins, these enzymes are found in the cytosol, where some, such as the oxidase and thiolase, are degraded

rapidly, while catalase accumulates and is degraded more slowly than in normal cells [86]. Among the consequences of defective peroxisomal assembly is a variety of abnormalities of morphogenesis. These are most notable externally in Zellweger syndrome, but abnormal neuronal migration takes place in neonatal adrenoleukodystrophy, as well as Zellweger syndrome [86, 87]. Abnormal migration is demonstrable in fetal tissue. Neurons normally found in the outer layers of the cerebral cortex are found in inner layers and in the white matter. Abnormal migration leads to microgyria and to thick pachygyria. Abnormal migration is not seen in rhizomelic chondrodysplasia punctata, and other peroxisomal disorders in which plasmalogen synthesis is defective, so this could not be the mechanism of the abnormal migration. On the other hand, VLCFA do not accumulate in the chondrodysplasias, so this could be involved in the abnormal neuronal pathogenesis [87]. Deficiency of plasmalogens makes cells sensitive to ultraviolet irradiation [88].

The subcellular localization of catalase correlates with the status of peroxisomes in histologic studies of tissues. In Zellweger patients, catalase is essentially all cytosolic, while in normal individuals as much as 65 percent of catalase sediments with the peroxisomal particles.

Defective peroxisomal function is manifest in pathways of plasmalogen synthesis, pipecolic acid, and phytanic metabolism, branched chain fatty acid oxidation and cholesterol metabolism. Plasma levels of VLCFA and bile acid intermediates are elevated. The VLCFA accumulate in this condition and in Zellweger syndrome because of failure to catabolize them [89]. All of the enzymes of peroxisomal β -oxidation are defective. These enzymes are synthesized normally, but they are degraded rapidly because they cannot target into peroxisomes. Cultured fibroblasts of a patient with neonatal adrenoleukodystrophy have been shown to make mRNA normally for an enzyme of fatty acid oxidation whose activity could not be found in autopsied liver [90].

Patients with Zellweger syndrome and neonatal adrenoleukodystrophy have dicarboxylic aciduria of predominantly medium-chain length [91], such as adipic (C6), suberic (C8), and sebacic (C10) acids. This reflects the failure of peroxisomal β -oxidation. The dicarboxylic aciduria may be modest compared with that seen in abnormalities of mitochondrial β -oxidation.

Levels of docosahexanoic acid (DHA) are low in brain, retina, liver, and plasma in patients with disorders of peroxisomal biogenesis [92, 93]. The mechanism is not yet clear, but may be a consequence of defective β -oxidation. DHA is important for the integrity of both brain and retina [94] and so may play a role in the pathogenesis of some clinical manifestations.

Bile acids are also metabolized to deoxycholic acid in peroxisomes, and precursors such as tri-hydroxycholestanoic acid (THCA) and dihydroxy-cholestanoic acid are present in high concentrations in patients [95]. This could relate to the pathogenesis of hepatic abnormality,

and levels of transaminases and bilirubin in plasma are regularly elevated in Zellweger patients.

The accumulation of pipecolic acid and its increased excretion in urine [44] is as the L-isomer. It appears to result from a failure to metabolize pipecolic acid to α -amino adipic acid which normally takes place in peroxisomes [96].

TREATMENT

No effective treatment has been developed for the disorders of peroxisome biogenesis. The dietary regimens under exploitation in X-linked adrenoleukodystrophy were explored in the milder examples of disorders of peroxisomal biogenesis. Improvement in a patient with neonatal adrenoleukodystrophy has been reported [97] following treatment with docosahexanoic acid (250 mg/day), but these observations have not generally been accepted. Clofibrate has been used without success to induce the formation of hepatic peroxisomes in Zellweger syndrome. Symptomatic therapy, such as the use of anticonvulsants, may be helpful in management.

REFERENCES

1. Ulrich H, Herschkowitz N, Heitz P *et al.* Adrenoleukodystrophy. Preliminary report of a congenital case. Light and electron microscopical immunohistochemical and biochemical findings. *Acta Neuropathol* 1978; **43**: 77.
2. Moser HW, Moser AB, Kawamura N *et al.* Adrenoleukodystrophy: elevated C26 fatty acid in cultured skin fibroblasts. *Ann Neurol* 1980; **7**: 542.
3. Jaffe R, Crumrine P, Hashida Y, Moser HW. Neonatal adrenoleukodystrophy. Clinical pathological and biochemical delineation of a syndrome affecting both males and females. *Am J Pathol* 1982; **108**: 100.
4. Moser HW, Moser AB, Kawamura N *et al.* Adrenoleukodystrophy: studies of the phenotype genetics and biochemistry. *Johns Hopkins Med J* 1980; **147**: 217.
5. Moser HW, Moser AB, Frayer KK *et al.* Adrenoleukodystrophy: increased plasma content of saturated very long chain fatty acids. *Neurology* 1981; **31**: 1241.
6. Haas HE, Johnson ES, Farrell DL. Neonatal-onset adrenoleukodystrophy in a girl. *Ann Neurol* 1982; **12**: 449.
7. Benke PJ, Reyes PF, Parker JC. New form of adrenoleukodystrophy. *Hum Genet* 1981; **58**: 204.
8. Mobley WC, White CL, Tennekoon G *et al.* Neonatal adrenoleukodystrophy. *Ann Neurol* 1982; **12**: 204.
9. Wolff J, Nyhan WL, Powell H *et al.* Myopathy in an infant with a fatal peroxisomal disorder. *Ped Neurol* 1986; **2**: 141.
10. Kelley RI, Datta NS, Dobyns WB *et al.* Neonatal adrenoleukodystrophy: new cases biochemical studies and differentiation from Zellweger and related peroxisomal polydistrophy syndromes. *Am J Med Genet* 1986; **23**: 869.

11. Aubourg P, Scotto J, Rocchiccioli F *et al.* Neonatal adrenoleukodystrophy. *J Neurol Neurosurg Psychiatry* 1986; **49**: 77.
12. Vamecq J, Draye J-P, Van Hoof F *et al.* Multiple peroxisomal enzymatic deficiency disorders. A comprehensive biochemical and morphological study of Zellweger cerebrohepatorenal syndrome and neonatal adrenoleukodystrophy. *Am J Pathol* 1986; **125**: 524.
13. Brul S, Westerveld A, Strijland A *et al.* Genetic heterogeneity in the cerebrohepatorenal (Zellweger) syndrome and other inherited disorders with a generalized impairment of peroxisomal function – a study using complementation analysis. *J Clin Invest* 1988; **81**: 1710.
14. Roscher AA, Hoefler S, Hoefler G *et al.* Genetic and phenotypic heterogeneity in disorders of peroxisome biogenesis – a complementation study involving cell lines from 19 patients. *Pediatr Res* 1989; **26**: 67.
15. Yajima S, Suzuki T, Shimozawa N *et al.* Complementation study of peroxisome-deficient disorders by immunofluorescence staining and characterization of fused cells. *Hum Genet* 1992; **88**: 491.
16. Shimozawa N, Tsukamoto T, Suzuki Y *et al.* A human gene responsible for Zellweger syndrome that affects peroxisome assembly. *Science* 1992; **255**: 1132.
17. Gartner J, Moser H, Valle D. Mutations in the 70K peroxisomal membrane protein gene in Zellweger syndrome. *Nat Genet* 1992; **1**: 16.
18. Dodi G, Braverman N, Wong C *et al.* Mutations in the PTS1 receptor gene PXR1 define complementation group 2 of the peroxisome biogenesis disorders. *Nat Genet* 1995; **9**: 115.
19. Gould SJ, Valle D. The genetics and cell biology of the peroxisome biogenesis disorders. *Trends Genet* 2000; **16**: 340.
20. Erdmann R, Wiebel FF, Flessau A *et al.* PAS1 a yeast gene required for peroxisome biogenesis encodes a member of a novel family of putative ATPases. *Cell* 1991; **64**: 499.
21. Reuber BE, Collins CS, Germain-Lee E *et al.* Mutations in PEX1 are the most common cause of Zellweger syndrome neonatal adrenoleukodystrophy and infantile Refsum disease. *Nat Genet* 1997; **17**: 445.
22. Cohen SMZ, Green WR, de la Cruz ZC *et al.* Ocular histopathological studies of neonatal and childhood adrenoleukodystrophy. *Am J Ophthalmol* 1983; **95**: 82.
23. Cohen SM, Brown FR III, Martyn L *et al.* Ocular histopathologic and biochemical studies of the cerebro-hepatorenal (Zellweger) syndrome and its relation to neonatal adrenoleukodystrophy. *Am J Ophthalmol* 1983; **96**: 488.
24. Brown RF III, McAdams AJ, Cummins JW *et al.* Cerebro-hepato-renal (Zellweger) syndrome and neonatal adrenoleukodystrophy: similarities in phenotype and accumulation of very long chain fatty acids. *Johns Hopkins Med J* 1982; **151**: 344.
25. Noetzel MJ, Clark HB, Moser HW. Neonatal adrenoleukodystrophy with prolonged survival. *Ann Neurol* 1983; **14**: 380.
26. Gootjes J, Elpeleg O, Eyskens F *et al.* Novel mutations in the PEX2 gene of four unrelated patients with a peroxisome biogenesis disorder. *Pediatr Res* 2004; **55**: 431.
27. Zellweger H. The cerebro-hepato-renal (Zellweger) syndrome and other peroxisomal disorders. *Dev Med Child Neurol* 1987; **29**: 821.
28. Torvik A, Torp S, Kase BE *et al.* Infantile Refsum's disease: a generalized peroxisomal disorder. Case report with postmortem examination. *J Neurol Sci* 1988; **85**: 39.
29. Budden SS, Kennaway NG, Buist NR *et al.* Dysmorphic syndrome with phytanic acid oxidase deficiency abnormal very long chain fatty acids and pipecolic acidemia: studies in four children. *J Pediatr* 1986; **108**: 33.
30. Weleber RG, Tongue AC, Kennaway NG *et al.* Ophthalmic manifestations of infantile phytanic acid storage disease. *Arch Ophthalmol* 1984; **102**: 1317.
31. Poll-Thá BT, Roels F, Ogier H *et al.* A new peroxisomal disorder with enlarged peroxisomes and a specific deficiency of acyl-CoA oxidase (pseudo-neonatal adrenoleukodystrophy). *Am J Hum Genet* 1988; **42**: 422.
32. Fournier B, Saudubray JM, Benichou B *et al.* Large deletion of the peroxisomal acyl-CoA oxidase gene in pseudoneonatal adrenoleukodystrophy. *J Clin Invest* 1994; **94**: 526.
33. Watkins PA, Chen WW, Harris CJ *et al.* Peroxisomal bifunctional enzyme deficiency. *J Clin Invest* 1989; **83**: 771.
34. van Grunsven EG, van Berkel E, Mooijer PAW *et al.* Peroxisomal bifunctional protein deficiency revisited: resolution of its true enzymatic and molecular basis. *Am J Hum Genet* 1999; **64**: 99.
35. Goldfischer S, Collins J, Rapin I *et al.* Deficiencies in several peroxisomal oxidative activities. *J Pediatr* 1986; **108**: 25.
36. van Grunsven EG, van Roermund CWT, Denis S, Wanders RJA. Complementation analysis of fibroblasts from peroxisomal fatty acid oxidation deficient patients shows high frequency of bifunctional enzyme deficiency plus intragenic complementation: unequivocal evidence for differential defects in the same enzyme protein. *Biochem Biophys Res Commun* 1997; **235**: 176.
37. Goldfischer S, Powers JM, Johnson AB *et al.* Striated adrenocortical cells in cerebro-hepato-renal (Zellweger) syndrome. *Virchows Arch* 1983; **401**: 355.
38. Manz HJ, Schuelein M, McCullough DC *et al.* New phenotypic variant of adrenoleukodystrophy: pathologic ultrastructural and biochemical study in two brothers. *J Neurol Sci* 1980; **45**: 245.
39. Singh I, Moser AB, Goldfischer S, Moser HW. Lignoceric acid is oxidized in the peroxisome: implications for the Zellweger cerebro-hepato-renal syndrome and adrenoleukodystrophy. *Proc Natl Acad Sci USA* 1984; **81**: 4203.
40. Goldfischer S, Collins J, Rapin I *et al.* Peroxisomal defects in neonatal onset and X-linked adrenoleuko-dystrophies. *Science* 1985; **227**: 67.
41. Moser AE, Singh I, Brown FR III *et al.* The cerebro-hepato-renal (Zellweger) syndrome and neonatal adrenoleukodystrophy: increased levels and impaired degradation of very long chain fatty acids and their use in prenatal diagnosis. *N Engl J Med* 1984; **310**: 1141.
42. Roscher A, Molzer B, Bernheimer H *et al.* The cerebrohepatorenal (Zellweger) syndrome: an improved method for the biochemical diagnosis and its potential value of prenatal detection. *Pediatr Res* 1985; **19**: 930.

43. Heymans HSA. Cerebro-hepato-renal (Zellweger) syndrome: clinical and biochemical consequences of peroxisomal dysfunction. Thesis, University of Amsterdam, 1984.
44. Danks DM, Tippet P, Adams C, Campbell P. Cerebro-hepato-renal syndrome of Zellweger: a report of eight cases with comments upon the incidence the liver lesion and fault in pipecolic acid metabolism. *J Pediatr* 1975; **86**: 382.
45. Moser HW, Moser AB, Powers JM *et al*. The prenatal diagnosis of adrenoleukodystrophy. Demonstration of increased hexacosanoic acid levels in cultured amniocytes and fetal adrenal gland. *Pediatr Res* 1982; **16**: 172.
46. Hajra AK, Datta NS, Jackson LJ *et al*. Prenatal diagnosis of Zellweger cerebrohepatorenal syndrome. *N Engl J Med* 1985; **312**: 445.
47. Wanders RJA, van Wijland MJA, van Roermund CWT *et al*. Prenatal diagnosis of Zellweger syndrome by measurement of very long chain fatty acid (C26:0) beta-oxidation in cultured chorionic villus fibroblasts: implications for early diagnosis of other peroxisomal disorders. *Clin Chim Acta* 1987; **165**: 303.
48. Rocchiccioli F, Aubourg P, Choiset A. Immediate prenatal diagnosis of Zellweger syndrome by direct measurement of very long chain fatty acids in chorionic villus cells. *Prenat Diagn* 1987; **7**: 349.
49. Wanders RJA, Schrakamp G, van den Bosch H *et al*. A prenatal test for the cerebro-hepatorenal (Zellweger) syndrome by demonstration of the absence of catalase-containing particles (peroxisomes) in cultured amniotic fluid cells. *Eur J Pediatr* 1986; **145**: 136.
50. Carey WF, Robertson EF, Van Cruyten C *et al*. Prenatal diagnosis of Zellweger's syndrome by chorionic villus sampling – and a caveat. *Prenat Diagn* 1986; **6**: 227.
51. Shimozawa N, Suzuki Y, Orii T *et al*. Standardization of complementation grouping of peroxisome-deficient disorders and the second Zellweger patient with peroxisomal assembly factor-1 (PAF-1) defect. *Am J Hum Genet* 1993; **52**: 843 (letter to the editor).
52. Goldfischer S, Moore CL, Johnson AB *et al*. Peroxisomal and mitochondrial defects in the cerebro-hepato-renal syndrome. *Science* 1973; **182**: 62.
53. Santos MJ, Imanaka T, Shio H *et al*. Peroxisomal membrane ghosts in Zellweger syndrome – aberrant organelle assembly. *Science* 1988; **239**: 1536.
54. Santos MJ, Imanaka T, Shio H, Lazarow PB. Peroxisomal integral membrane proteins in control and Zellweger fibroblasts. *J Biol Chem* 1988; **263**: 10502.
55. Aikawa J, Chen WW, Kelley RI *et al*. Low-density particles (W-particles) containing catalase in Zellweger syndrome and normal fibroblasts. *Proc Natl Acad Sci USA* 1991; **88**: 10084.
56. Santos M, Imanaka T, Shio H *et al*. Peroxisomal membrane ghosts in Zellweger syndrome – aberrant organelle assembly. *Science* 1988; **239**: 1536.
57. Santos MJ, Hoefler S, Moser AB *et al*. Peroxisome assembly mutations in humans: structural heterogeneity in Zellweger syndrome. *J Cell Physiol* 1992; **151**: 103.
58. Gould SJ, Keller GA, Subramani S. Identification of peroxisomal targeting signals located at the carboxy terminus of four peroxisomal proteins. *J Cell Biol* 1988; **107**: 897.
59. Subramani S. Protein translocation into peroxisomes. *J Biol Chem* 1996; **271**: 32483.
60. Terlecky SR, Legakis JE, Hueni SE, Subramani S. Quantitative analysis of peroxisomal protein import *in vitro*. *Exp Cell Res* 2001; **263**: 98.
61. Geisbrecht BV, Collins CS, Reuber BE, Gould SJ. Disruption of a PEX1-PEX6 interaction is the most common cause of the neurologic disorders Zellweger syndrome neonatal adrenoleukodystrophy and infantile Refsum disease. *Proc Natl Acad Sci USA* 1998; **95**: 8630.
62. Tamura S, Shimozawa N, Suzuki Y *et al*. A cytoplasmic AA family peroxin Pex1p interacts with Pex6p. *Biochem Biophys Res Commun* 1998; **245**: 883.
63. Maxwell MA, Nelson PV, China SJ *et al*. A common PEX1 frameshift mutation in patients with disorders of peroxisome biogenesis correlates with the severe Zellweger syndrome phenotype. *Hum Genet* 1999; **105**: 38.
64. Preuss N, Brosius U, Biermanns M *et al*. PEX1 mutations in complementation group 1 of Zellweger spectrum patients correlate with severity of disease. *Pediatr Res* 2002; **51**: 706.
65. Wanders RJA, Mooijer PAW, Dekker C *et al*. Disorders of peroxisome biogenesis: complementation analysis shows genetic heterogeneity with strong overrepresentation of one group (PEX1 deficiency). *J Inher Metab Dis* 1999; **22**: 314.
66. Collins CS, Gould SJ. Identification of a common mutation in severely affected PEX1-deficient patients. *Hum Mutat* 1999; **14**: 45.
67. Rosewich H, Ohlenbusch A, Gartner J. Genetic and clinical aspects of Zellweger spectrum patients with PEX1 mutations. *J Med Genet* 2005; **42**: e58.
68. Crane DI, Maxwell MA, Paton BC. PEX1 mutations in the Zellweger spectrum of the peroxisome biogenesis disorders. *Hum Mutat* 2005; **26**: 167.
69. Masuno M, Shimozawa N, Suzuki Y *et al*. Assignment of the human peroxisome assembly factor-1 gene (PXMP3) responsible for Zellweger syndrome to chromosome 8q211 by fluorescence *in situ* hybridization. *Genomics* 1994; **20**: 141.
70. Tsukamoto T, Mijura S, Fujiki Y. Restoration by a 35K membrane protein of peroxisome assembly in a peroxisome-deficient mammalian cell mutant. *Nature* 1991; **350**: 77.
71. Thieringer R, Raetz CRH. Peroxisome-deficient. Chinese hamster ovary cells with point mutations in peroxisome assembly Factor-1*. *J Biol Chem* 1993; **268**: 12631.
72. Steinberg SJ, Fensom AH. Complementation analysis in patients with the clinical phenotype of a generalised peroxisomal disorder. *J Med Genet* 1996; **33**: 295.
73. Stanley WA, Filipp FV, Kursula P *et al*. Recognition of a functional peroxisome type 1 target by the dynamic import receptor Pex5p. *Molec Cell* 2006; **24**: 653.
74. Warren DS, Morrell JC, Moser HW *et al*. Phenotype-genotype relationships in PEX10-deficient peroxisome biogenesis disorder patients. *Hum Mutat* 2000; **15**: 509.
75. Gould SJ, Kalish JE, Morrell JC *et al*. Pex13p is an SH3 protein of the peroxisome membrane and a docking factor for the predominantly cytoplasmic PTS1 receptor. *J Cell Biol* 1996; **135**: 85.

76. Shimozawa N, Suzuki Y, Zhang Z *et al.* Nonsense and temperature-sensitive mutations in PEX13 are the cause of complementation group H of peroxisome biogenesis disorders. *Hum Mol Genet* 1999; **8**: 1077.
77. Braverman N, Steel G, Lin P *et al.* PEX7 gene structure alternative transcripts and evidence for a founder haplotype for the frequent RCDP allele 1292ter. *Genomics* 2000; **63**: 181.
78. Chang C-C, Warren DS, Sacksteder KA, Gould SJ. PEX12 interacts with PEX5 and PEX10 and acts downstream of receptor docking in peroxisomal matrix protein import. *J Cell Biol* 1999; **147**: 761.
79. South ST, Sacksteder KA, Li X *et al.* Inhibitors of COPI and COPII do not block PEX3-mediated peroxisome synthesis. *J Cell Biol* 2000; **149**: 1345.
80. Honsho M, Tamura S, Shimozawa N *et al.* Mutation in PEX16 is causal in the peroxisome-deficient Zellweger syndrome of complementation group D. *Am J Hum Genet* 1998; **63**: 1622.
81. Matsuzono Y, Kinoshita N, Tamura S *et al.* Human PEX19: cDNA cloning by functional complementation mutation analysis in a patient with Zellweger syndrome and potential role in peroxisomal membrane assembly. *Proc Natl Acad Sci USA* 1999; **96**: 2116.
82. Matsumoto N, Tamura S, Furuki S *et al.* Mutations in novel peroxin gene PEX26 that cause peroxisome-biogenesis disorders of complementation group 8 provide a genotype-phenotype correlation. *Am J Hum Genet* 2003; **73**: 233.
83. Matsumoto N, Tamura S, Fujiki Y. The pathogenic peroxin Pex26p recruits the Pex1p-Pex6p AAA ATPase complexes to peroxisomes. *Nature Cell Biol* 2003; **5**: 454.
84. Matsumoto N, Tamura S, Furuki S *et al.* Mutations in novel peroxin gene PEX26 that cause peroxisome-biogenesis disorders of complementation group 8 provide a genotype-phenotype correlation. *Am J Hum Genet* 2003; **73**: 233.
85. Weller S, Cajigas I, Morrell J *et al.* Alternative splicing suggests extended function of PEX26 in peroxisome biogenesis. *Am J Hum Genet* 2005; **76**: 987.
86. Wanders RJA, Los M, Roest B *et al.* Activity of peroxisomal enzymes and intracellular distribution of catalase in Zellweger syndrome. *Biochem Biophys Res Commun* 1984; **123**: 1054.
87. Powers JM, Tummons RC, Caviness VS Jr *et al.* Structural and chemical alterations in the cerebral maldevelopment of fetal cerebro-hepato-renal (Zellweger) syndrome. *J Neuropathol Exp Neurol* 1989; **48**: 270.
88. Hoefler G, Paschke E, Hoeffler S *et al.* Photosensitized killing of cultured fibroblasts from patients with peroxisomal disorders due to pyrene fatty acid-mediated ultraviolet damage. *J Clin Invest* 1991; **88**: 1873.
89. Suzuki Y, Orii T, Mori M *et al.* Deficient activities and proteins of peroxisomal beta-oxidation enzymes in infants with Zellweger syndrome. *Clin Chim Acta* 1986; **156**: 191.
90. Chen WW, Watkins PA, Osumit T *et al.* Peroxisomal beta-oxidation enzyme proteins in adrenoleukodystrophy: distinction between X-linked adrenoleukodystrophy and neonatal adrenoleukodystrophy. *Proc Natl Acad Sci USA* 1987; **84**: 1435.
91. Pampols T, Ribes A, Pineda M *et al.* Medium chain dicarboxylic and hydroxydicarboxylic aciduria in a case of neonatal adrenoleukodystrophy. *J Inherit Metab Dis* 1987; **10**: 217.
92. Martinez M. Abnormal profiles of polyunsaturated fatty acids in the brain liver kidney and retina of patients with peroxisomal disorders. *Brain Res* 1993; **583**: 171.
93. Martinez M. Severe deficiency of docosahexaenoic acid in peroxisomal disorders. A defect of delta-4 desaturations? *Neurology* 1990; **40**: 1292.
94. Bazan NG. Supply of n-3 polyunsaturated fatty acids and their significance in the central nervous system. In: Wurtman RJ, Wurtman JJ (eds). *Nutrition and the Brain*, vol. 8. New York: Raven, 1990: 1.
95. Hanson RF, Szczepanick-Van Leeuwen P, Williams GC *et al.* Defects of bile acid synthesis in Zellweger's syndrome. *Science* 1979; **203**: 1107.
96. Wanders RJA, Romeyn GJ, van Roermund CWT *et al.* Identification of L-pipecolate oxidase in human liver and its deficiency in the Zellweger syndrome. *Biochem Biophys Res Commun* 1988; **154**: 33.
97. Martinez M, Pineda M, Vidal R, Martin B. Docosahexaenoic acid: a new therapeutic approach to peroxisomal patients. Experience with two cases. *Neurology* 1993; **43**: 1389.

DISORDERS OF PURINE METABOLISM

64.	Lesch-Nyhan disease and variants	483
65.	Adenine phosphoribosyl-transferase deficiency	498
66.	Phosphoribosylpyrophosphate synthetase and its abnormalities	503
67.	Adenosine deaminase deficiency	507
68.	Adenylosuccinate lyase deficiency	514
69.	Orotic aciduria	518

Lesch-Nyhan disease and variants

Introduction	483	Treatment	494
Clinical abnormalities	483	References	494
Genetics and pathogenesis	490		

MAJOR PHENOTYPIC EXPRESSION

Impaired motor development, spastic cerebral palsy, involuntary movements; self-injurious behavior; hyperuricemia, uricosuria, urinary tract calculi, nephropathy, tophi, gouty arthritis; and deficient activity of hypoxanthine guanine phosphoribosyl transferase (HPRT). In variants, hyperuricemia, gout, or renal calculi; in a neurologic variant, the phenotype is identical to that of Lesch-Nyhan disease, but self-mutilation is absent and intelligence may be normal; in another variant, the expression is of dystonia mimicking spastic diplegia and mildly impaired mental development. Variant HPRT enzymes may have activity that is 0 or as much as 50 percent of normal in hemolysates, but over 1.4 percent of control in the intact cell assay.

INTRODUCTION

Lesch-Nyhan disease was first described in 1964 [1] as a syndrome in which disordered purine metabolism, as exemplified by hyperuricemia, uric aciduria, increased turnover of an enlarged uric acid pool, and enormous overproduction of purine *de novo* was associated with a neurological picture of athetoid cerebral palsy and bizarre,

compulsive, self-mutilative behavior. The overproduction of purine from an intravenous glycine precursor was 20 times the normal value [2], whereas in adults with gouty arthritis the largest rates observed were twice the normal value. The hallmark feature of the behavior was loss of tissue because of biting. The gene was recognized early from pedigree studies to be situated on the X chromosome [3], and transmission is usually as a fully recessive character. There have been a few affected females, most reflecting a nonrandom inactivation of the normal X chromosome. The enzyme defect in hypoxanthine guanine phosphoribosyl transferase (HPRT) (Figure 64.1) was discovered in 1967 by Seegmiller and colleagues [4]. The gene was cloned in 1982 by Friedmann and colleagues [5, 6]. A large number and variety of mutations have been defined [7–10].

CLINICAL ABNORMALITIES

Male infants with the Lesch-Nyhan disease appear normal at birth and usually develop normally for the first six to eight months. The first sign is usually the appearance of orange crystals which give the appearance of orange sand in the diapers [11], and this history is regularly obtained from the parents. This manifestation of crystalluria would permit early diagnosis, prior to the development of

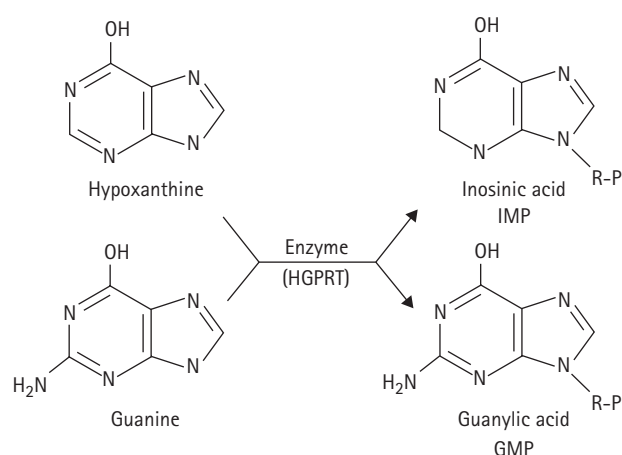


Figure 64.1 Hypoxanthine-guanine phosphoribosyl transferase (HPRT), the site of the defect in the Lesch-Nyhan disease.

neurologic or behavioral features, and I am pleased to say that we have now encountered patients who were detected early on this basis. Hematuria or urinary tract stones, as well as crystalluria, may develop during the early months of life.

Defective motor development usually becomes evident in the second six months of life. Commonly, it is the failure to reach developmental milestones or poor head control that brings the infant to attention. At this age, hypotonia is commonly evident, but some patients may have hypertonic lower extremities. The classic patient with Lesch-Nyhan disease does not learn to walk and must have some support even to sit unaided (Figure 64.2). Patients learn to sit in a chair if they are fastened securely about the chest, as well as the waist, and narrow wheelchairs are preferred (Figure 64.3). This is the preferred daytime situation for virtually all patients.

Involuntary movements have been seen in 100 percent of our patients [11]. Extrapyramidal features indicate abnormality in the function of the basal ganglia [12, 13]. Varying descriptions in the literature probably reflect varying use of the language, rather than variation in the neurologic phenotype. Increasingly, we have recognized dystonia as a major feature with its extensive cocontraction of agonist and antagonist muscles, dystonic posturing especially on intention, and overflow muscular contractions elsewhere. There may be flailing movements of the extremities that have been called 'ballismus'. Choreic movements are particularly common with excitement,



Figure 64.2 MJ: A seven-year-old boy with the Lesch-Nyhan syndrome illustrating the motor disability as exemplified by an inability to sit without support, dystonic posturing in the position of the upper extremities, spasticity, and a left spontaneous Babinski.



Figure 64.3 OK: A Lesch-Nyhan patient in the typical position. Supported properly, the patient can be part of the action and he can get around in a wheelchair.

either emotionally positive or negative. We have also observed fine, typically athetoid movements (Figure 64.2). Opisthotonic spasms, retrocollis, or periodic arching of the back are characteristic. They begin in infancy but continue even into adult life, becoming in some patients a semivoluntary component of behavior. Among pyramidal features spasticity is usually considerable. Scissoring of the lower extremities is common. The increased muscle pull leads ultimately to dislocation of both hips in most patients. One of our patients had dislocated both patellae proximally. Many patients develop contractures, predominantly in flexion. Deep tendon reflexes are increased, but reflexes may be difficult to obtain because excitement engendered by the examination leads to so much activity. Babinski reflexes are regularly found, but may be absent. Spontaneous dorsiflexion of the toe (Figure 64.2) may be easier to observe than one elicited by a detailed neurologic examination. Dysfunction of ocular motor activity was reported [13] in 15 patients with severe deficiency of HPRT and the extrapyramidal manifestation of the classic Lesch-Nyhan phenotype. In contrast, in seven neurologically normal patients with lesser deficiencies of HPRT, ocular motility was normal. In the former group, ocular motor apraxia was characterized by interruption of fixation by frequent unwanted saccades toward minor visual distractions. Even the most cooperative patients could not fixate on a central target for a minute. Voluntary saccades were initiated by sudden head movement, an eye blink, or both. Immobilization of the head led to a delay in initiated voluntary saccades. Four patients had blepharospasm and

two had ocular tics. These abnormalities were deemed consistent with abnormalities in the function of the basal ganglia or their connections with ocular motor centers. Seizures occur in some patients, but are not a major feature of the disease, and the electroencephalogram is usually normal.

Impaired mental development is a prominent but controversial feature of the disease. In many patients, the IQ as tested has approximated 50. However, adequate testing is very difficult because the behavior and a short attention span get in the way of the testing. Clearly, these patients have cognitive abilities well above the level of the motor disability. A few patients have been observed in whom there has been normal or near normal intelligence. Many parents feel that their sons are intellectually normal. Two of our patients were doing grade level work in normal high schools. These patients were toilet trained. Few of the others we have studied were. All of them learn to speak, but dysarthria makes their speech difficult to understand. Despite the gravity of the cerebral disease, there is little evidence that this is a progressive, degenerative disease, but diminished neurologic function with age has been observed in adult variants (see below).

Behavioral features are integral to the disease and the behavior is compulsive and aggressive. Self-injurious behavior occurs in 100 percent of patients with the classic Lesch-Nyhan syndrome [11]. On the other hand, there are exceptions to every rule. Puig *et al.* [14] have reported two patients, one six years of age, but the other 21 years, who have not mutilated despite having uncles, and in one case a brother, who displayed the complete phenotype including



Figure 64.4 MJ: The degree of the mutilation of the lip is relatively mild.



Figure 64.5 JJ: A 14-year-old boy, illustrating an extreme degree of mutilation around the face.

self-injurious behavior. More recently, we have reported [15] a family in which a single mutation has been expressed as three different phenotypes, only one of whom had the classic Lesch-Nyhan phenotype. Two of these adults manifested deterioration of motor functions in adulthood, such that the ability to walk was lost.

The behavior is compulsive and aggressive. While aggressive activity is predominantly directed against the patients themselves, they do attempt to injure others and sometimes succeed, but the motor disability largely prevents much success. The most characteristic feature is self-destructive biting of the lips (Figures 64.4 and 64.5) and fingers (Figures 64.6, 64.7, and 64.8). Unlike many



Figure 64.6 Freshly bitten lesions of the thumb and forefinger of a six-year-old Saudi boy with the Lesch-Nyhan syndrome.



Figure 64.7 This same boy as illustrated in Figure 64.6 managed to bite his great toe as well. He also had loss of tissue from biting the lower lip, banged his head and created sores on his chin by rubbing it on the floor.



Figure 64.8 MJ: Roentgenogram of the left hand, illustrating partial amputation of the fifth finger.

other patients with mental impairment who engage in self-mutilative behavior, these patients bite with a ferocity that leads to significant loss of tissue. Partial amputation of fingers has been observed (Figure 64.8).

The differential diagnosis of self-injury of this disease includes the De Lange syndrome and dysautonomia. Self-injurious behavior has also been observed in as many as 58 percent of boys with fragile X syndrome [16]. The behavior consisted predominately of biting fingers or the dorsum of the hand or hitting the head. Information on severity, for example loss of tissue or amputations, was not provided. In patients with Prader-Willi syndrome [17, 18], the behavior consisted of picking the skin over an extensive body area. In Smith-Magenis syndrome, self-injurious behavior was described [19] as near universal. Hand biting was the most common behavior in these patients too, but they also manifested some unusual behaviors, including: picking at fingers or toenails until they bled, onychotillomania, and even complete removal

of the nail, and polyembolokoilamania (the inserting of foreign objects into orifices of the body). It is thought that in this disorder there may be peripheral neuropathy. The behavioral phenotype of Smith-Magenis syndrome also includes extreme disturbance of sleep and a 'self-hugging' stereotype. The differential diagnosis does not really include sensory neuropathy and indifference to pain. Those patients tend to look like pugilists and the injuries are accidental. Lesch-Nyhan patients do not have sensory abnormalities; they scream in pain when they bite themselves and cry in terror of its anticipation. As patients become older, they learn to become aggressive with speech. Four-letter Anglo-Saxon expressions are common. Males appear to have a considerable interest in the opposite sex. This sometimes leads to inappropriate groping; more often it leads to frustration.

Apraxic discoordination of the lips and tongue make feeding difficult and swallowing is imperfect. In addition, most have required some teeth extraction in order to protect against damage by biting. Patients with this disease feed poorly. Virtually all vomit, and this, too, seems to be incorporated into the behavior. In most patients, growth in height and weight are well below the norms for chronological age [11]. Autopsy studies have revealed no consistent abnormalities in the brain and a number of brains have been judged to be normal. So too have routine neuroimaging studies. However, quantitative magnetic resonance imaging (MRI) comparison of patients and age-matched controls has revealed a 34 percent decrease in the volume of the caudate [20], which appeared to reflect abnormal development as opposed to atrophy.

Hyperuricemia is present in virtually all patients. The concentration of uric acid in the plasma is usually between 9 and 12 mg/dL, which level is at the limit of solubility of urate in plasma. Patients with some degree of acute or chronic glomerular insufficiency may have higher concentrations of uric acid, and some who are very efficient at excreting urate may have lower values, occasionally in the normal range. The clinician must be careful, however, at accepting a conclusion that a plasma uric acid is normal from a laboratory whose norms were established on adult males in whom hyperuricemia is common. All patients excrete large amounts of uric acid in the urine. Twenty-four-hour excretions of 600–1000 mg are the rule in patients weighing 15 kg or more. Throughout childhood, patients with this disease excrete three to four times as much uric acid as do control individuals of comparable size. In relation to body weight, they excrete 40–70 mg of uric acid per kg. Another pitfall in interpreting uric acid data arises from the propensity of microorganisms to consume purines including uric acid; conditions of collection of a 24-hour sample at room temperature are ideal for bacterial purposes. For this reason, it is best to avoid collecting 24-hour samples, except for research purposes under which each sample is added to the batch in the freezer as soon as obtained. For diagnostic purposes, it is more convenient to collect a fresh sample and analyze



Figure 64.9 Uric acid crystals in the sediment from a fresh, centrifuged sample of urine viewed through polarized light. (Reprinted with permission from Stapleton FB and Linshaw MA. *N Engl J Med* 1994; 330: 762.)

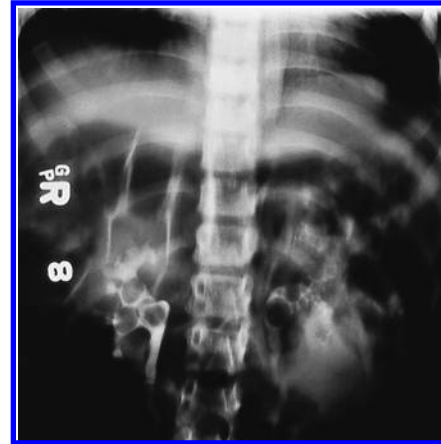


Figure 64.11 Intravenous pyelogram of a patient with the Lesch-Nyhan disease. There were numerous radiolucent calculi.

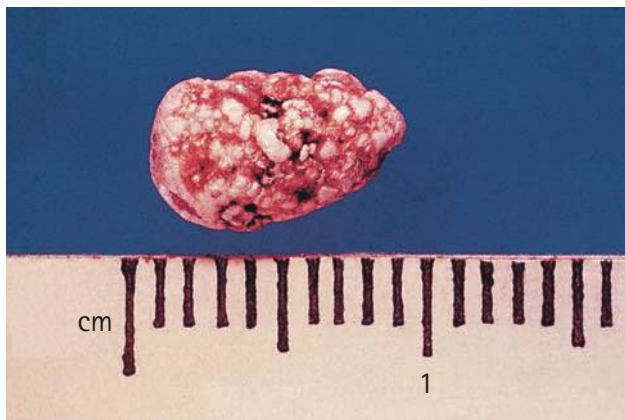


Figure 64.10 Urinary calculus recovered from the urine of a patient with hypoxanthine guanine phosphoribosyl transferase (HPRT) deficiency.



Figure 64.12 A 17-year-old boy with prominent tophaceous deposits in the ears. The violaceous inflammatory reaction is unusual around tophi. It subsided following treatment with colchicine.

promptly for uric acid and creatinine [21]. These patients regularly excrete 3 to 4 mg of uric acid/mg of creatinine, while in control individuals older than one year of age the level is less than 1.

The clinical consequences of the accumulation of large amounts of uric acid in body fluids are manifestations classic for gout. These patients pass large quantities of urate crystals in the urine (Figure 64.9). Episodes of hematuria and crystalluria are the rule and may cause abdominal pain. Urinary tract calculi are regularly observed (Figures 64.10 and 64.11) and they may occur as early as the first months of life; they lead regularly to urinary tract infections. In the absence of treatment, urate nephropathy develops as a result of the deposition of sodium urate in the renal parenchyma. Death from renal failure at less than ten years of age was the expected outcome before the development



Figure 64.13 Roentgenogram of an adult patient with hypoxanthine guanine phosphoribosyl transferase (HPRT) deficiency illustrating typical lesions of chronic tophaceous gout.

of allopurinol. Tophi may be seen in those unusual patients who survive without treatment beyond ten years (Figure 64.12). Acute gouty arthritis is even more rare, but has occurred uniformly in untreated patients reaching adult life. Chronic tophaceous gout has been observed (Figure 64.13) [22].

Sudden unexplained death has been relatively common in older patients with classic Lesch-Nyhan disease. We and others have speculated that forced retrocollis and cervical cord injury might be responsible.

The discovery of the enzyme defect in Lesch-Nyhan disease was followed shortly by the recognition of deficiency of the enzyme in patients with gout [23] or urinary tract calculi [24]. Initial expectations that these populations might be quite large have turned out not to be true, and most patients with abnormal HPRT enzymes have classic Lesch-Nyhan disease. Nevertheless, a certain number of variants has been described – enough that assay for HPRT activity should be performed in any patient with overproduction hyperuricemia, and any hyperuricemic patient with a diagnosis of cerebral palsy; especially if the prenatal and perinatal course were normal. In an infant found to have HPRT deficiency, the distinction between the classic Lesch-Nyhan prognosis and that of the variant forms is of major importance.

The populations of variant patients initially described [23, 24] had hyperuricemia, with gout or renal stones and HPRT enzyme activity greater than zero. For this reason, they have been referred to as partial variants. However, additional experience shows that some of these patients have zero activity in the erythrocyte lysate assay and cannot in this way be distinguished from patients with Lesch-Nyhan disease. The phenotype of the patient with the classic partial variant enzyme consists of manifestations that can be directly related to the accumulation of uric acid in body fluids. The central nervous system and behavior have been described as normal. However, recent observations suggest that this may be simplistic [25]. The earliest presentation, as in the Lesch-Nyhan disease, should be orange sand in the diaper. Advantages of the earliest diagnosis possible should be effective therapy to prevent renal complications. Renal calculi have been observed even early in childhood [24]. Such patients may present with hematuria, colic, urinary tract infection, or passage of a stone (Figures 64.10 and 64.11). There may be acute obstruction of one or both ureters and hydronephrosis. Crystalluria is so massive that an intercurrent infection that leads to vomiting or dehydration may result in complete obstruction of the ureters with sludge requiring emergency surgery and ureteral lavage. We have observed this complication in which the crystals were of uric acid, but also of oxypurinol [26], the oxidation product of allopurinol. In the initial evaluation of follow up of a patient with overproduction hyperuricemia, renal ultrasound or an intravenous pyelogram is essential to assess for the presence of uric acid or xanthine stones, which are radiolucent. A radiopaque stone in a patient with hyperuricemia indicates the codeposition of calcium salts. Ultrasonography (Figure

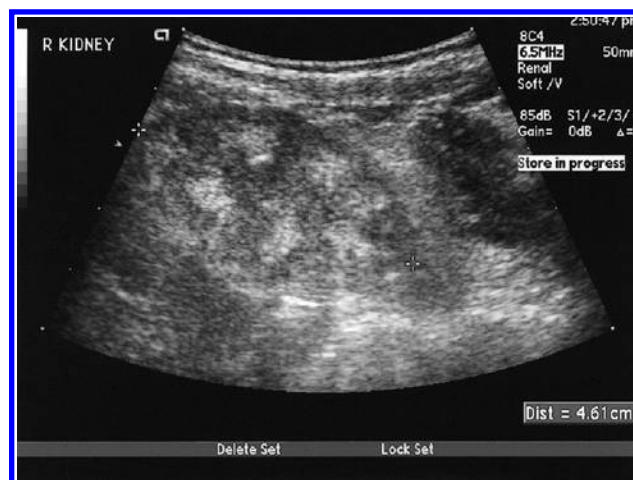


Figure 64.14 Ultrasonograph of the kidneys of an infant with hypoxanthine guanine phosphoribosyl transferase (HPRT) deficiency. Uric acid crystals are echogenic.



Figure 64.15 TL: A seven-year-old boy with a variant enzyme hypoxanthine guanine phosphoribosyl transferase (HPRT) whose initial presentation was with painless hematuria. The initial episode was thought to be hemorrhagic cystitis, but recurrent episodes suggested chronic glomerulonephritis. Biopsy was normal. HPRT activity was 3 percent in the erythrocyte assay at high concentration of substrates.

64.14) may reveal echogenic crystals in the substance of the kidney. Untreated accumulation of uric acid can lead to urate nephropathy and renal failure.

Another presentation is with painless hematuria in a pattern that suggests a diagnosis of hemorrhagic cystitis or glomerulonephritis (Figure 64.15) [27]. Cystourethrography in this patient led to transient hypertension, oliguria, and

azotemia; following anesthesia and renal biopsy, oliguria/anuria recurred, and the BUN (blood, urea, nitrogen) rose to 80 mg/dL.

A majority of patients with partial variants have presented with gout [23, 28, 29]. Acute attacks of gouty arthritis and tophi usually occur first in adult life even though the hyperuricemia has been present since birth. It appears to require approximately 20 years of hyperuricemia before the conditions are appropriate for precipitation of the needle-like crystals of urate that produce the inflammatory response of the acute attack of arthritis. There are exceptions to every rule and we have found a variant enzyme in a patient in whom acute attacks of arthritis began at one year.

Behavior is normal in these variant patients (Figure 64.16). Antisocial behavior that has rarely been described may be completely unrelated to the defect in HPRT [30].

A quite different phenotype is what we have called the neurologic variant [31]. This picture, which has been observed in a small, but important group of patients, is characterized by a neurological examination that is identical to that of the classic Lesch-Nyhan patient. These patients are generally diagnosed as having cerebral palsy or athetoid cerebral palsy. They are confined to wheelchairs and unable to walk. The index patient was reported by Catel and Schmidt [32], as a patient with the Lesch-Nyhan syndrome in whom intelligence and behavior were normal. He has since been followed by Manzke and colleagues [33], and we had the opportunity to study him just after his graduation from university (Figure 64.17). He spoke



Figure 64.16 RL: A young man with a partial variant hypoxanthine guanine phosphoribosyl transferase (HPRT), was diagnosed after he passed a uric acid calculus during his second episode of renal colic. He and three male relatives displayed approximately 5 percent of the control level of HPRT by erythrocyte assay.

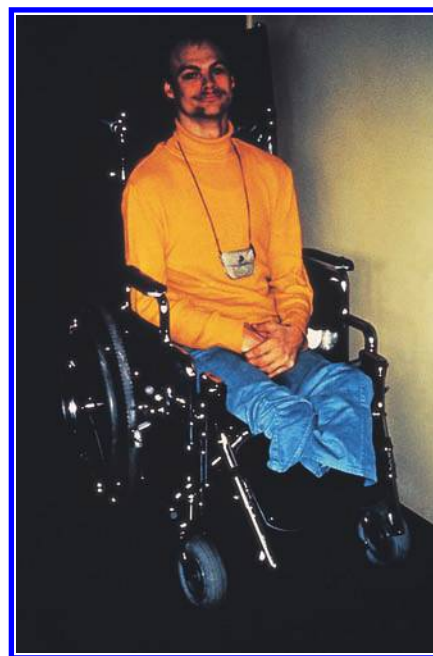


Figure 64.17 HCB: A 21-year-old patient with the classic neurologic variant [34]. Neurologic manifestations were identical to those of the Lesch-Nyhan disease, but intelligence and behavior were normal. A variant enzyme was documented by the intact cell assay.

English and German. There were no abnormalities of intelligence or behavior. His variant HPRT was zero in erythrocyte lysates, but activity was readily distinguished from the Lesch-Nyhan disease by the intact cell assay [34]. Activity approached 10 percent of control, and there was enough activity to permit kinetic studies [35]. A maternal uncle had had a similar syndrome. Other patients have since been studied in whom variant enzymes have produced this phenotype [36, 37]. Behavior is normal and intelligence is normal or nearly normal.

Virtually complete deficiency of HPRT in erythrocyte lysates was recently reported [38] in a 28-year-old patient with hyperuricemia and gout, along with deficiency of glucose-6-phosphate dehydrogenase, who had mild developmental impairment, mild dysarthria, and hypotonia. Puig and colleagues [14] observed a variant patient who was intellectually normal, but had such spasticity and was so dystonic that he could not walk.

Another phenotype has been observed in a family with an HPRT variant that we have called HPRT^{Salamanca} [39]. In this pedigree, four males in three generations had an identical phenotype, the most prominent feature of which was referred to as spastic diplegia. They were all able to walk, albeit with a spastic gait. The boy we studied (Figures 64.18 and 64.19) wore out the tops of the toes of his shoes because of the way he dragged his feet. Muscle tone and deep tendon reflexes were much more increased in the legs than in the arms. Babinski responses were positive bilaterally. There was a bilateral pes cavus and exaggerated



Figure 64.18 AA: A boy with HPRT^{Salamanca}. He had a pronounced spastic diplegia and a mild degree of developmental impairment.



Figure 64.19 AA: Rear view walking illustrating the dragging of his toes.

lumbar lordosis. Mental impairment was mild. Involved members of the family were effectively employed as migrant grape pickers in Southern France. The proband had developed tophaceous gout by 32 years of age. Each of the involved members of the family had clinodactyly of the fifth fingers and proximally placed thumbs. The

abnormal enzyme displayed approximately 8 percent of control activity in the whole cell assay. Other abnormalities observed in Lesch-Nyhan patients, such as megaloblastic anemia or testicular dysfunction, may be seen in variants. Many variant individuals have shown a considerable interest in the opposite sex. Some have married.

We made a distinction early on as to two and then three different phenotypes of the HPRT deficiency. Now it is clear that there are at least four. Neurological variants have been usefully distinguished between those who can and those who cannot walk [14]. With increasing experience, the distinctions begin to seem artificial and there is now at least one mutation reported in which three different phenotypes were seen in a single family [15]. The main argument for the distinction is the issue of prognosis in an infant newly diagnosed as having HPRT deficiency. This is a major issue for parents.

GENETICS AND PATHOGENESIS

The molecular defect in the Lesch-Nyhan disease is in the activity of the enzyme hypoxanthine-guanine phosphoribosyl transferase (E.C.2.4.2.8.) (Figure 64.1). This enzyme catalyzes the reaction of hypoxanthine or guanine with phosphoribosyl pyrophosphate (PRPP) to form their respective nucleotides, inosinic, and guanylic acids. The enzyme is present in all cells of the body. It is particularly active in basal ganglia and testis. The defect is readily detectable in erythrocyte hemolysates and in cultured fibroblasts. In the erythrocyte, quantitative assays reveal no activity in patients with classic phenotype.

HPRT is determined by a gene on the long arm of the X chromosome at Xq26-27 (Figure 64.20). The disease is transmitted as an X-linked recessive trait (Figure 64.21). It is essentially a disease of the male, occurring at a frequency of approximately one in 380,000 births. Six females have been observed [40, 41].

The heterozygous carrier can be detected by assay of the enzyme in individual cells, such that the two populations of cells specified in the Lyon hypothesis are demonstrated. This has been accomplished by cloning or pharmacologic selection in thioguanine or azaguanine. A more convenient, but still tedious, method is hair root analysis [42], which takes advantage of the largely clonal nature of individual hair follicles, but requires the plucking of at least 30 individual hairs and analysis of enzyme activity in each one. Definition of the molecular defect in an individual family permits direct testing for carriers of the mutation. In families in which the mutation is known, it is the method of choice. It can be simplified especially where a new restriction site is created or an old one eliminated by the mutation. In informative families, linkage analysis of restriction fragment length polymorphism (RFLP) has been used for heterozygote detection [43]. Study of the carrier status of mothers has indicated that the incidence of a mutation is considerably less than the one-third of

affected patients predicted by population genetics theory for an X-linked lethal gene [44]. There is some evidence for an effect of paternal age and of mutation occurring in the genesis of the carrier mother. Simple testing of erythrocytes or leukocytes for enzyme activity is not useful for carrier detection, because activity in heterozygotes is virtually always normal [44], an index probably of selection against mutant cells.

Prenatal diagnosis has regularly been accomplished by assay of the enzyme in amniocytes [45–48] or chorionic villus samples, and a considerable number of affected and nonaffected fetuses has been detected. Nucleotidase activity, which is high in villus material, may lead to the breakdown of newly formed IMP and thus cause a false-positive diagnosis of an affected fetus [47] so it is important to inhibit the nucleotidase during the assay. Among normal individuals, a certain amount of RFLP has been identified and the linkage appears to be quite tight. This has permitted its use in informative families in which the mother carries identifiable alleles for prenatal diagnosis, as well as for the detection of heterozygosity [43]. In families in which the mutation is known, determination of its presence or absence is the method of choice for prenatal diagnosis.

The gene for the HPRT enzyme has been cloned and its nucleotide sequence determined [5, 6]. It spans more than 44 kb of DNA; its coding region contains 654 nucleotides in nine exons. The mRNA is 1.6 kb, but the reading frame approximates 700 bases and the enzyme contains 217 amino acids [49], which forms a tetramer.

The introduction of the polymerase chain reaction has permitted the identification of a number of mutations in patients with HPRT deficiency [7, 8, 50–56]. In the most recent summary, 302 mutations were recorded [7, 8]. In most families studied, a unique mutation has been found. The same mutation has only rarely been found in unrelated pedigrees. In most patients, the gene is not grossly altered; on the other hand, in classic Lesch-Nyhan patients, the results of the mutation have been major ones leading to essentially no activity of the enzyme. In eight classic Lesch-Nyhan phenotypes [50], an entire exon was deleted in two; and in three, nonsense mutations led to a stop mutation and hence a markedly truncated protein. In Lesch-Nyhan patients, some large deletions have been detectable by Southern blot analysis [56]. The number of coding sequence deletions in the latest update numbered 66 [8]. In classic Lesch-Nyhan patients, a complete spectrum of mutations has been observed including major disruptions, such as deletions, insertions, frame shift mutations leading to exon skipping and stop codons leading to truncated unstable proteins which are readily degraded [7]. Missense mutations were classically nonconservative in the classic phenotype; for instance, an aspartic acid for a glycine at position 16, a leucine for a phenylalanine at position 74, and a tyrosine for an aspartic acid at position 201. A very small number of CpG mutational hot spots were identified, for instance at arginine 51 and 170 of the HPRT protein. These are thought to represent deamination of

5-methylcytosine to thymine changing the message for arginine to stop [57]. This mutation has occurred 11 times among reported patients [7, 51]. Among other mutations of the gene, duplications have been reported, one of which resulted from recombination of Alu sequences in introns 6 and 8 [58]. This patient, though 22 years and with severely impaired mental development, had no self-injurious behavior; so he could represent a variant.

In contrast to the mutations in the classic Lesch-Nyhan disease, the majority of the variants had missense mutations. Most had a single nucleotide change. Among seven variant patients reported from Spain [14], none had mutations that predicted altered protein size. Of the 302 mutations summarized by Jinnah *et al.* [7, 8], there were two deletions in variant patients in contrast to six in Lesch-Nyhan patients. The one insertion observed in a variant added a single amino acid and did not alter the reading frame. Changes, such as a leucine to valine at codon 78, appeared relatively conservative and led to a variant phenotype whereas a leucine to glutamine change at 78 led to a Lesch-Nyhan phenotype [7]. In HPRT^{Salamanca}, there were two mutations: a T to G change at position 128 and a G to A at 130. These led to conversions of two adjacent amino acids at positions 43 and 44 in the protein: a methionine to an arginine and an aspartic acid to an asparagine. These alterations appear nonconservative. However, they illustrate another issue; mutations in the variants have tended to cluster in the amino end of the molecule. In contrast, Lesch-Nyhan alterations in this area tend to be those with stop codons. Mutations of HPRT that alter RNA splicing have been summarized by O'Neill and colleagues [59]. They accounted for 12.5 percent of all known mutations, those of human disease and those induced by mutagens. These are interesting because they have pleiotropic effects leading to multiple RNA products. These authors also pointed out that harmless polymorphisms have not been described in the HPRT gene.

In sum, it is clear that major interruptions of the gene and truncations of the protein lead to classic Lesch-Nyhan disease. Most missense mutations lead also to the classic phenotype, but among variants virtually all have missense mutations or frame shifts.

Prenatal diagnosis and heterozygote detection can readily be carried out in a family in which the mutation is known. In one family [60], a nonsense mutation of the CpG site for arginine 169 was identified in a fetus and five female heterozygotes, in three of whom X chromosomal mosaicism could not be demonstrated by repeated hair root analysis or by selection of fibroblasts in azaguanine and thioguanine. Presumably, this represents an extreme example of selection against the mutant cell. We recommend prenatal diagnosis in pregnancies in which a mother has had an affected infant even if she is found not to be a heterozygote in order to avoid the problem of gonadal mosaicism. Also, since there have now been documented affected females, we recommend prenatal assessment of HPRT status even when the fetus is found to have two X chromosomes.

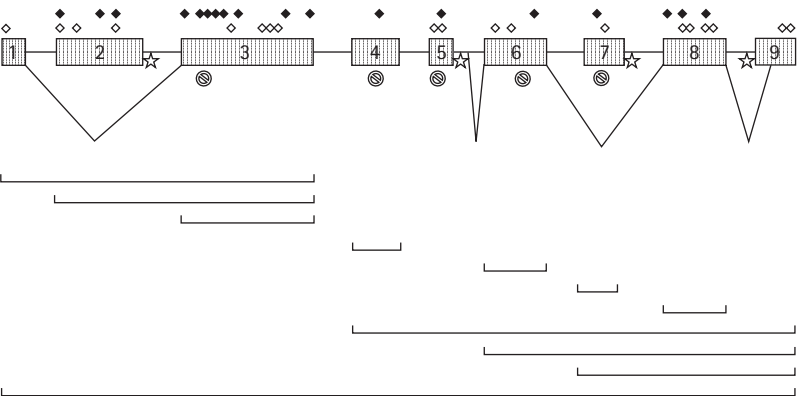


Figure 64.20 The hypoxanthine guanine phosphoribosyl transferase (HPRT) gene. The nine exons and sites of mutations are shown. The open diamonds, variants; filled diamonds, classic Lesch-Nyhan patients; stars, frame shifts; stops signs, stop codons. Deletions of various sizes are shown below, virtually all in Lesch-Nyhan patients.

Establishment of the diagnosis in a patient with the typical phenotype requires determination that the activity of HPRT in a hemolysate is zero. However, some patients with different variants and different clinical phenotypes may also have zero activity in the erythrocyte assay. Assay of the enzyme in intact cultured fibroblasts has permitted the distinction of these populations: patients with Lesch-Nyhan disease have activity that is less than 1.4 percent of normal [61]; the variants have all had more activity.

Patients with variant forms of abnormal HPRT can also be characterized as different from normal by the assay [62–64] of the enzyme in erythrocytes which identifies the presence of enzyme deficiency. Families in which there is a considerable amount of activity in the erythrocyte are also distinguishable from classic Lesch-Nyhan variants. There may be 1 percent, 5 percent, 15 percent, or more of the normal level of activity; and in a kindred in which there are a number of involved members (Figure 64.20) [62], each involved member has the same deficiency. All four males in one kindred (Figures 64.16 and 64.22) had 5 percent of control activity. In other families, altered kinetics may be illustrated by different activity at saturating concentrations of substrates than at low concentrations; therefore, we routinely carry out the assay under these two conditions. In one of our patients with gout, the erythrocyte activity was 60 percent of control [62].

On the other hand, the difficulty arises because a number of patients with phenotypes very different from the Lesch-Nyhan disease have been found to have no activity of HPRT as measured in erythrocyte or fibroblast lysates. This is a likely result of the fact that structurally abnormal enzymes are often unstable, and activity disappears rapidly once cell walls are broken. It is for this reason that no correlation has been found between the level of activity of the enzyme in hemolysates and clinical features in patients [63]. This is not because patients with the Lesch-Nyhan disease display any appreciable activity in these assays; it is because patients with quite mild phenotypes, including no central nervous system abnormality, also have no activity.

This problem was solved by the development of the more physiological method in which enzyme activity is assessed in intact cells [65, 66]. Cultured fibroblasts are incubated with ¹⁴C-hypoxanthine, the products are separated by high performance liquid chromatography, and the total number of picomoles of isotope incorporated into purine compounds is expressed per nanomole of total purine compounds present [35]. The method permits the determination of kinetic properties of the enzyme. The Km for hypoxanthine found in normal fibroblasts was identical

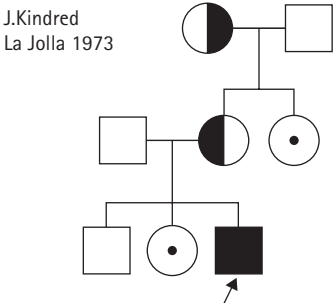


Figure 64.21 Pedigree of MJ. Symbols employed were black box (hemizygous male), half black circle (heterozygous female), and circle with a dot (a female tested and found to be normal).

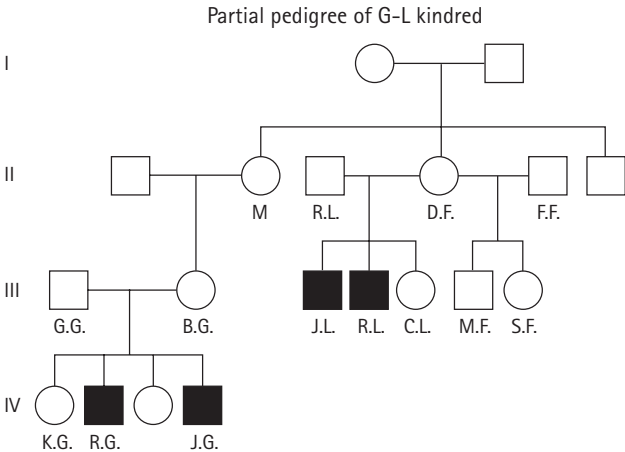


Figure 64.22 Partial pedigree of the G-L kindred (Figure 64.16) [24].

to that of purified human HPRT, and a number of kinetic variants have been documented [66]. The patient illustrated in Figure 64.17 was shown with this method [34] to have a variant different from the classic Lesch-Nyhan enzyme; his HPRT converted 9 percent of ^{14}C -hypoxanthine and 27 percent of ^{14}C -guanine to products that were mostly adenine and guanine nucleotides. In a series of patients with varying phenotypes, a roughly inverse correlation was obtained between enzyme activity and clinical severity (Figure 64.23) [35]. The activity obtained with hypoxanthine correlated better than did that obtained with guanine, and for this reason we routinely carry out the assay with hypoxanthine substrate. In this analysis, Lesch-Nyhan patients have displayed activity below 1.2–1.4 percent of normal, and the classic partial variants all had greater than 10 percent of control activity. The neurologic variants have had intermediate levels of activity. Patients with HPRT^{Salamanca} had 7.3 percent of control activity. Torres and Puig [67] have employed an intact erythrocyte assay with similar results. They have referred to the classic Lesch-Nyhan patients as type 4 and those with no neurologic findings as type 1. Neurologic variants type 2 and 3 were distinguished by the fact that in type 2 they could walk.

Variant enzymes have been observed to have a variety of other properties that have aided their characterization as distinct, such as unusual sensitivity to fluoride [68], unusual kinetic properties, or altered heat stability [23, 24]. Electrophoretic analysis has revealed mobilities that

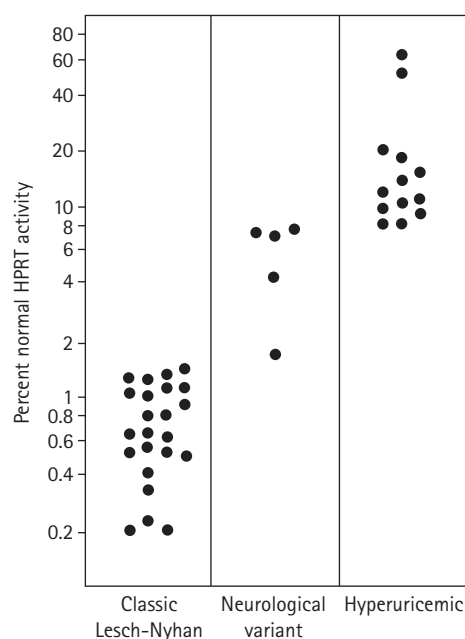


Figure 64.23 Activity of hypoxanthine guanine phosphoribosyl transferase (HPRT) in intact fibroblasts. The level of enzyme activity was roughly inversely proportional to the degree of clinical severity. Actually, the values fell into three groups, correlated with phenotype: the Lesch-Nyhan, the neurologic variant, and the classic partial variant.

were both faster and slower than the normal enzyme [63]. Antibody generated against normal human HPRT has demonstrated the presence [62] and the absence [69] of cross-reacting material (CRM). In all the variants studied, the activity of adenine phosphoribosyl transferase (APRT) is increased; the level may approximate 150 percent of control.

When the primary structure of normal human HPRT was determined [70], it became possible to determine the amino acid substitutions in variants in which a substantial amount of activity permitted purification and the preparation of peptide maps. For instance, in HPRT^{Toronto}, the substitution of glycine for arginine at position 50 is far removed from the binding sites for substrate, and, consistent with this, the kinetics were normal, and the phenotype was that of a classic hyperuricemic variant. In similar fashion, the transposition in HPRT^{London} was far removed from substrate binding sites.

Overproduction of purines via the *de novo* pathway is probably largely a consequence of an accumulation of phosphoribosyl-pyrophosphate (PRPP). Increased amounts of uric acid accumulating in body fluids lead to the clinical manifestations of gout and its renal complications. It is important to remember that normals for routine chemistry laboratories in general hospital are established for adults. A serum concentration of 5 mg/dL in an infant or child is distinctly elevated [71]. The degree of overproduction in the variants is the same as in the Lesch-Nyhan disease. It is as if the production of purines has been reset at maximum or at a similar high plateau; they all have the same elevation in the amounts of uric acid in the urine and in the blood.

In the presence of defective activity of HPRT, concentrations of PRPP rise, and there may be diminished feedback inhibition of glutamine amidotransferase by purine nucleotides. The rate of synthesis of purines via the *de novo* pathway increases markedly as studied *in vivo* with labeled glycine. Concentrations of hypoxanthine have been found to be elevated in the cerebrospinal fluid; levels were four-fold greater than those of control individuals [72]. Concentrations of uric acid are not elevated in the spinal fluid.

The pathogenesises of the cerebral and behavioral features of Lesch-Nyhan disease are not clear, while those features that are shared with patients with gout are clearly consequences of the accumulation of uric acid. Substantial evidence indicates that there is an imbalance of cerebral neurotransmitters. The best evidence for altered dopaminergic function came from the postmortem study of the brains of three patients in which there was statistically significant depression of dopaminergic function in the caudate, putamen, nucleus accumbens, and external pallidum [73].

The concentration of dopamine was decreased in the caudate nucleus of the brains of two more patients with Lesch-Nyhan disease [74]. In these brains, immunoreactive D₁ and D₂ dopamine receptors were increased in the putamen. Increase in these receptors has also been

observed in positron emission tomography (PET) studies in Parkinson disease [75]. These findings support a theory of dopamine supersensitivity in Lesch-Nyhan disease [76].

Levels of homovanillic acid (HVA) in the spinal fluid were found to be low [77]. Studies of PET with ligands specific for targets in the basal ganglia revealed reduction in binding to dopamine uptake transporters by 73 percent in the putamen and 56 percent in the caudate [78]. Another study showed over 60 percent reduction in fluorodopa uptake [79]. Further evidence for neurotransmitter imbalance was the transient cessation of self-injurious behavior following treatment with the serotonin precursor, 5-hydroxytryptophan with carbidopa [80].

HPRT is not directly included in the formation of dopamine, but its role in the formation of guanine-nucleotides, especially GTP, the substrate for the first and rate-limiting step in the synthesis of tetrahydrobiopterin (BH_4), could make for decrease in dopamine because BH_4 is the cofactor for tyrosine hydroxylase. Thus, limited local supply of GTP could lead to depletion of BH_4 and of dopamine. In fact, HPRT-deficient mice, which have low levels of striatal dopamine, have been found to have statistically significantly lowered striatal BH_4 [81]. Treatment of these mice with BH_4 did not correct the dopamine deficiency in mutant mice. Man is not a mouse and treatment was confined to a single day. In addition, Manzke and colleagues [82] treated a patient with a variant HPRT, in which the phenotype lacked abnormal behavior, with 17.5 mg/kg of BH_4 over 1 day. Levels of HVA and HIAA in the cerebrospinal fluid, which were very low prior to treatment rose to normal with treatment; L-dopa plus carbidopa and 5-hydroxytryptophan were given at the same time. The same treatment was given to a nine-year-old patient with classic Lesch-Nyhan disease and severe spasticity, who was reported to improve unequivocally, but the effect was lost after 5 weeks. It is unclear how much of the reported changes were related to BH_4 .

In studies of adenosinergic systems in brain in HGPRT knockout mice Bertelli *et al.* [83] found increased expression of the ADORA 1A adenosine receptor gene and a mild decrease in ADORA 2A expression.

TREATMENT

Allopurinol has been effective in reducing concentrations of uric acid and alleviating all of its direct clinical consequences. Doses of 200–400 mg/day lead promptly to normal plasma concentrations. Calculi and tophi are prevented or resorbed as concentrations of uric acid and in blood and urine fall. Nephropathy and arthritis are prevented. The total production of purine does not change; concentrations of xanthine and hypoxanthine increase. Some patients develop xanthine calculi. Determination of the levels of these oxypurines may be useful in providing the optimal dose of allopurinol. We aim to maximize the content of hypoxanthine without running the risk of

oxypurinol lithiasis. It has become clear that doses required to accomplish this are highly individual.

Dietary approaches to reduction of purine and uric acid output by reducing intake of purine-rich foods are ineffective. Similarly, feeding purines does not increase it. The production level appears set by the metabolism. On the other hand, these patients are virtually all thin and predominantly short. The enormous loss of nitrogen and preformed purine in the urine, along with dysphagia and vomiting, make keeping up nutrition difficult. A diet high in protein and calories appears prudent. The occurrence of megaloblastic anemia and the requirement of folate containing cofactors at two steps in purine synthesis would make the provision of folate prudent via folate rich foods or a supplement.

Pharmacologic approaches to therapy based on the neurotransmitter imbalance have not yet been successful, but this is a promising direction, possibly aided by PET and the demonstration of a reduction in dopamine transporters [78]. The availability of the cloned normal gene raises the possibility of gene therapy. Transfection of Lesch-Nyhan cells *in vitro* has been demonstrated, along with expression of normal enzyme [84]. Long-term expression *in vivo* remains an objective.

The only successful approaches to the self-injurious behavior have been the removal of teeth and physical restraint. Tooth removal can be selective. In addition to physical restraint it is useful to be imaginative in finding ways to encourage purposeful activity to replace self-injurious behavior [85]. For instance, on entering an automobile, one adult patient in a community placement regularly managed to leave a hand in place to be caught when the door was closed. His caretaker learned to avoid this problem by the simple expedient of asking the patient to close the door himself.

REFERENCES

1. Lesch M, Nyhan WL. A familial disorder of uric acid metabolism and central nervous system function. *Am J Med* 1964; **36**: 561.
2. Nyhan WL. Introduction – clinical features. In: *Seminars on the Lesch-Nyhan Syndrome*. (Bland JH (ed.)). *Fed Proc* 1968; **27**: 1027.
3. Nyhan WL, Pesek J, Sweetman L *et al.* Genetics of an X-linked disorder of uric acid metabolism and cerebral function. *Pediatr Res* 1967; **1**: 5.
4. Seegmiller JE, Rosenbloom FM, Kelley WN. Enzyme defect associated with sex-linked human neurological disorder and excessive purine synthesis. *Science* 1967; **155**: 1682.
5. Jolly DJ, Esty AC, Bernard HU, Friedmann T. Isolation of a genomic clone encoding human hypoxanthine guanine phosphoribosyl transferase. *Proc Natl Acad Sci USA* 1982; **79**: 5038.
6. Jolly DL, Okayama H, Berg P *et al.* Isolation and characterization of a full length, expressible cDNA for human hypoxanthine guanine phosphoribosyl transferase. *Proc Natl Acad Sci USA* 1983; **80**: 477.

7. Jinnah HA, De Gregorio L, Harris JC *et al*. The spectrum of inherited mutations causing HPRT deficiency: 75 new cases and a review of 196 previously reported cases. *Mutat Res* 2000; **463**: 309.
8. Jinnah HA, Harris JC, Nyhan WL, O'Neill JP. The spectrum of mutations causing HPRT deficiency: an update. *Nucleosides Nucleotides Nucleic Acids* 2004; **23**: 1153.
9. Yamada Y, Nomura N, Yamada K, Wakamatsu N. Molecular analysis of HPRT deficiencies: an update of the spectrum of Asian mutations with novel mutations. *Mol Genet Metab* 2007; **90**: 70.
10. Torres RJ, Puig JG. The diagnosis of HPRT deficiency in the 21st century. *Nucleosides Nucleotides Nucleic Acids* 2008; **27**: 564.
11. Christie R, Bay C, Kaufman LA *et al*. Lesch-Nyhan disease: clinical experience with nineteen patients. *Dev Med Child Neurol* 1982; **24**: 293.
12. Jinnah HA, Harris JC, Reich SG *et al*. The motor disorder of Lesch-Nyhan disease. *Mov Disord* 1998; **13**: 98.
13. Jinnah HA, Harris JC, Rothstein JD *et al*. Ocular motor dysfunction in Lesch-Nyhan disease. *Ped Neurol* 2001; **24**: 200.
14. Puig JG, Torres RJ, Mateos FA *et al*. The spectrum of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency. Clinical experience based on 22 patients from 18 Spanish families. *Medicine* 2001; **80**: 102.
15. Hladnik U, Nyhan WL, Bertelli M. Variable expression of HPRT deficiency in 5 members of a family with the same mutation. *Arch Neurol* 2008; **65**: 1240.
16. Symons FJ, Clark RD, Hatton DD *et al*. Self-injurious behavior in young boys with fragile X syndrome. *Am J Med Genet* 2003; **118A**: 115.
17. Dykens EM, Kasari C. Maladaptive behavior in children with Prader-Willi syndrome, Down syndrome, and nonspecific mental retardation. *Am J Ment Retard* 1997; **102**: 228.
18. Symons FJ, Butler MG, Sanders MD *et al*. Self-injurious behavior and Prader-Willi syndrome: Behavioral forms and body locations. *Am J Ment Retard* 1999; **104**: 260.
19. Finucane B, Dirrigl KH, Simon EW. Characterization of self-injurious behaviors in children and adults with Smith-Magenis syndrome. *Am J Ment Retard* 2001; **106**: 52.
20. Harris JC, Lee RR, Jinnah HA *et al*. Craniocerebral magnetic resonance imaging measurement and findings in Lesch-Nyhan syndrome. *Arch Neurol* 1998; **55**: 547.
21. Kaufman JM, Greene ML, Seegmiller JE. Urine uric acid to creatinine ratio: screening test for disorders of purine metabolism. *J Pediatr* 1968; **73**: 583.
22. Nieto LH, Nyhan WL, Page T *et al*. Syndrome de Lesch-Nyhan: nueva variante con actividad de hipoxantina-guanina fosforribosil transferasa (HPRT) superior a la de la enfermedad clasica y deteccion del rasgo heterocigoto en los hematies de la portadora. *Med Clin* 1985; **84**: 68.
23. Kelley WL, Greene ML, Rosenbloom FM *et al*. Hypoxanthine-guanine phosphoribosyltransferase deficiency in gout: a review. *Ann Intern Med* 1969; **70**: 155.
24. Kogut MD, Donnell GN, Nyhan WL, Sweetman L. Disorder of purine metabolism due to partial deficiency of hypoxanthine guanine phosphoribosyltransferase. *Am J Med* 1970; **48**: 148.
25. Jinnah HA, Ceballos-Picot I, Torres RJ *et al*. Attenuated variants of Lesch-Nyhan disease. *Brain* 2010; **133**: 671.
26. Landgrebe AR, Nyhan WL, Coleman M. Urinary tract stones resulting from the excretion of oxypurinol. *N Engl J Med* 1975; **292**: 626.
27. Sweetman L, Hoch MA, Bakay B *et al*. A distinct human variant of hypoxanthine-guanine phosphoribosyltransferase. *J Pediatr* 1978; **92**: 385.
28. Henderson JF, Kelley WN, Rosenbloom FM, Seegmiller JE. Inheritance of purine phosphoribosyltransferase in man. *Am J Hum Genet* 1969; **21**: 61.
29. Wilson JM, Young AB, Keelly WN. Hypoxanthine-guanine phosphoribosyltransferase deficiency. The molecular basis of the clinical syndromes. *N Engl J Med* 1983; **309**: 900.
30. Benke PJ, Herrick H. Azaguanine-resistance as a manifestation of a new form of metabolic overproduction of uric acid. *Am J Med* 1972; **52**: 547.
31. Nyhan WL. Inborn errors of purine metabolism. In: Cockburn F, Gitzelmann R (eds). *Inborn Errors of Metabolism in Humans*, Monograph based upon Proceedings of the International Symposium held in Interlaken, Switzerland, September 2-5, 1980. Lancaster: MTP Press, 1982: 13-36.
32. Catel VW, Schmidt J. Uber familiar gichtich Diathese in Verbindung mit zerebralen und renale Symptomen bei einem Kleinkind. *Dtsch Med Wochenschr* 1959; **84**: 2145.
33. Manzke H, Harms D, Dormer K. Zur Problematic der Behandlung der kongenitalen Hyperurikamie. *Monatsschr Kinderheilkd* 1971; **119**: 424.
34. Bakay B, Nissinen E, Sweetman L *et al*. Utilization of purines by an HPRT variant in an intelligent, nonmutilative patient with features of the Lesch-Nyhan syndrome. *Pediatr Res* 1979; **13**: 1365.
35. Page T, Bakay B, Nissinen E, Nyhan WL. Hypoxanthine-guanine phosphoribosyltransferase variants: correlation of clinical phenotype with enzyme activity. *J Inher Metab Dis* 1981; **4**: 203.
36. Gottlieb RP, Koppel MM, Nyhan WL *et al*. Hyperuricaemia and choreoathetosis in a child without mental retardation or self-mutilation: a new HPRT variant. *J Inher Metab Dis* 1982; **5**: 183.
37. Hersh JH, Page T, Hand ME *et al*. Clinical correlations in partial hypoxanthine guanine phosphoribosyltransferase deficiency. *Pediatr Neurol* 1986; **2**: 302.
38. Micheli V, Jacomeli G, Notarantonio L *et al*. Purine and pyrimidine metabolism in a patient with virtually complete HPRT deficiency but not Lesch-Nyhan syndrome. *European Society for Study of Purine and Pyrimidine Metabolism in Man*. 2001: 95.
39. Page T, Nyhan WL, Morena de Vega V. Syndrome of mild mental retardation spastic gait, and skeletal malformations in a family with partial deficiency of hypoxanthine-guanine phosphoribosyltransferase. *Pediatrics* 1987; **79**: 713.
40. Ogasawara N, Yamada Y, Goto H. HPRT gene mutations in a female Lesch-Nyhan patient. In: Harkness RA *et al*. (eds). *Purine and Pyrimidine Metabolism in Man VII, Part B*. New York: Plenum Press, 1991: 109.
41. DeGregorio L, Nyhan WL, Serafin E, Chamoles NA. An unexpected affected female patient in a classical Lesch-Nyhan family. *Mol Genet Metab* 2000; **69**: 263.

42. Page T, Bakay B, Nyhan WL. An improved procedure for the detection of hypoxanthine-guanine phosphoribosyltransferase heterozygotes. *Clin Chem* 1982; **28**: 4181.
43. Nussbaum RL, Crowder WE, Nyhan WL, Caskey CT. A three-allele restriction-fragment-length polymorphism at the hypoxanthine phosphoribosyltransferase locus in man. *Proc Natl Acad Sci USA* 1983; **80**: 4035.
44. Francke U, Felsenstein J, Gartler SM *et al*. The occurrence of new mutants in the X-linked recessive Lesch-Nyhan disease. *Am J Hum Genet* 1976; **28**: 123.
45. Alford RL, Redman JB, O'Brien WE, Caskey CT. Lesch-Nyhan syndrome: carrier and prenatal diagnosis. *Prenat Diagn* 1995; **15**: 329.
46. Bakay B, Francke U, Nyhan WL, Seegmiller JE. Experience with detection of heterozygous carriers and prenatal diagnosis of Lesch-Nyhan disease. In: Muller MM, Kaiser E, Seegmiller JU (eds). *Purine Metabolism in Man II: Regulation of Pathways and Enzyme Defects*. New York: Plenum Press, 1977: 351.
47. Page T, Broock RL. A pitfall in the prenatal diagnosis of Lesch-Nyhan syndrome by chorionic villus sampling. *Prenatal Diagn* 1990; **10**: 153.
48. Nyhan WL, Vuong L-UC, Broock R. Prenatal diagnosis of Lesch-Nyhan disease. *Prenatal Diagn* 2003; **23**: 807.
49. Wilson JM, Tarr GE, Mahoney WC, Kelley WN. Human hypoxanthine-guanine phosphoribosyltransferase. *J Biol Chem* 1982; **257**: 10978.
50. Sege-Peterson K, Chambers J, Pate T *et al*. Characterization of mutations in phenotypic variants of hypoxanthine phosphoribosyltransferase deficiency. *Hum Mol Genet* 1992; **1**: 427.
51. Gibbs RA, Nguyen P, McBride LJ *et al*. Identification of mutations leading to the Lesch-Nyhan syndrome by automated direct DNA sequencing of *in vitro* amplified cDNA. *Proc Natl Acad Sci USA* 1990; **86**: 1919.
52. Gibbs RA, Nguyen P, Edwards A *et al*. Multiplex DNA deletion detection and exon sequencing of the hypoxanthine phosphoribosyltransferase gene in Lesch families. *Genomics* 1990; **7**: 235.
53. Davidson BL, Tarle SA, Palella TD, Kelley WN. Molecular basis of hypoxanthine-guanine phosphoribosyltransferase deficiency in ten subjects determined by direct sequencing of amplified transcripts. *J Clin Invest* 1989; **84**: 342.
54. Davidson BL, Tarle SA, van Antwerp M *et al*. Identification of 17 independent mutations responsible for human hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency. *Am J Hum Genet* 1991; **48**: 951.
55. Tarle SA, Davidson BL, Wu VC *et al*. Determination of the mutations responsible for the Lesch-Nyhan syndrome in 17 subjects. *Genomics* 1991; **10**: 499.
56. Yang TP, Patel PI, Stout JT *et al*. Molecular evidence for new mutations in the HPRT locus in Lesch-Nyhan patients. *Nature* 1984; **310**: 412.
57. Ehrlich M, Wang RY. 5-Methylcytosine in eukaryotic DNA. *Science* 1981; **212**: 1350.
58. Marcus S, Hellgren D, Lambert B *et al*. Duplications in the hypoxanthine phosphoribosyltransferase gene caused by Alu-Alu recombination in a patient with Lesch-Nyhan syndrome. *Hum Genet* 1993; **90**: 477.
59. O'Neill JP, Finette BA. Transition mutations at CpG dinucleotides are the most frequent *in vivo* spontaneous single-based substitution mutation in the human HPRT gene. *Environ Mol Mutagen* 1998; **32**: 188.
60. Marcus S, Steen AM, Andersson B *et al*. Mutation analysis and prenatal diagnosis in a Lesch-Nyhan family showing non-random X-inactivation interfering with carrier detection tests. *Hum Genet* 1992; **89**: 395.
61. Page T, Bakay B, Nissinen E, Nyhan WL. Hypoxanthine-guanine phosphoribosyltransferase variants: correlation of clinical phenotype with enzyme activity. *J Inher Metab Dis* 1981; **4**: 203.
62. Sweetman L, Borden M, Lesch P *et al*. Diminished affinity for purine substrates as a basis for gout with mild deficiency of hypoxanthine guanine phosphoribosyl transferase. In: Muller MM, Kaiser E, Seegmiller JE (eds). *Purine Metabolism in Man: II. Regulation of Pathways and Enzyme Defects*. New York: Plenum Press, 1977: 325.
63. Emmerson BT, Thompson L. The spectrum of hypoxanthine guanine phosphoribosyl transferase deficiency. *Q J Med* 1973; **166**: 423.
64. Bakay B, Nissinen E, Sweetman L. Analysis of radioactive and nonradioactive purine bases, nucleosides, nucleotides by high-speed chromatography on a single column. *Anal Biochem* 1978; **86**: 65.
65. Bakay B, Nissinen EA, Sweetman L, Nyhan WL. Analysis of radioactive and nonradioactive purine bases, purine nucleosides and purine nucleotides by high-speed chromatography on a single column. *Monographs in Human Genetics*, vol. 10. Basel: Karger, 1978: 127.
66. Page T, Bakay B, Nyhan WL. Kinetic studies of normal and variant hypoxanthine phosphoribosyltransferases in intact fibroblasts. *Anal Biochem* 1982; **122**: 144.
67. Torres RJ, Puig JG. The diagnosis of HPRT deficiency in the 21st century. *Nucleosides Nucleotides Nucleic Acids* 2008; **7**: 564.
68. Sweetman L, Hoch MA, Bakay B *et al*. A distinct human variant of hypoxanthine-guanine phosphoribosyltransferase. *J Pediatr* 1978; **92**: 385.
69. Bakay B, Becker MA, Nyhan WL. Reaction of antibody to normal human hypoxanthine phosphoribosyltransferase with products of mutant genes. *Arch Biochem Biophys* 1976; **177**: 415.
70. Arnold WJ, Kelley WN. Molecular basis of hypoxanthine guanine phosphoribosyltransferase purification and subunit structure. *J Biol Chem* 1971; **246**: 7398.
71. Monkus E St J, Nyhan WL, Fogel BJ, Yankow S. Concentrations of uric acid in the serum of neonatal infants and their mothers. *Am J Obstet Gynecol* 1970; **108**: 91.
72. Sweetman L. Urinary and cerebrospinal oxypurine levels and allopurinol metabolism in the Lesch-Nyhan syndrome. *Fed Proc* 1968; **27**: 1055.
73. Lloyd KG, Hornykewicz O, Davidson L *et al*. Biochemical evidence of dysfunction of brain neurotransmitters in the Lesch-Nyhan syndrome. *N Engl J Med* 1981; **305**: 1106.

74. Saito Y, Ito M, Hanaoka S *et al*. Dopamine receptor upregulation in Lesch-Nyhan syndrome: a postmortem study. *Neuropediatrics* 1999; **30**: 66.
75. Brooks DJ, Ibanex V, Sawle GV *et al*. Striatal D2 receptor status in patients with Parkinson's disease, striatonigral degeneration, and progressive supranuclear palsy, measured with 11C-raclopride and positron emission tomography. *Ann Neurol* 1992; **31**: 184.
76. Breese GR, Criswell HE, Duncan GE, Mueller RA. Dopamine deficiency in self-injurious behavior. *Psychopharmacol Bull* 1989; **25**: 353.
77. Silverstein FS, Johnston JV, Hutchinson RJ, Edwards NL. Lesch-Nyhan syndrome: CSF neurotransmitter abnormalities. *Neurology* 1985; **35**: 907.
78. Wong DF, Harris JC, Naidu S *et al*. Dopamine transporters are markedly reduced in Lesch-Nyhan disease *in vivo*. *Proc Natl Acad Sci USA* 1996; **93**: 5539.
79. Ernst M, Zametkin AJ, Matochik JA *et al*. Presynaptic dopaminergic deficits in Lesch-Nyhan disease. *N Engl J Med* 1996; **334**: 1568.
80. Nyhan WL, Johnson HG, Kaufman IA, Jones KL. Serotonergic approaches to the modification of behavior in the Lesch-Nyhan syndrome. *Appl Res Mental Retard* 1980; **1**: 25.
81. Hyland K, Kasim S, Egami K. Tetrahydrobiopterin deficiency and dopamine loss in a genetic mouse model of Lesch-Nyhan disease. *J Inherit Metab Dis* 2004; **27**: 165.
82. Manzke H, Gustmann H, Koke HG, Nyhan WL. Hypoxanthine and tetrahydrobiopterin treatment of a patient with features of the Lesch-Nyhan syndrome. In: Nyhan WL, Thompson LF, Watts RWE (eds). *Purine and Pyrimidine Metabolism in Man V. Part A: Clinical Aspects Including Molecular Genetics*. Advances in Experimental Medicine and Biology, vol. 195 A. New York: Plenum Publishing, 1986: 197.
83. Bertelli M, Cecchin S, Lapucci C. Study of the adenosinergic system in the brain of HPRT knockout mouse (Lesch-Nyhan disease). *Clin Chim Acta* 2006; **373**: 104.
84. Miller AD, Jolly DJ, Friedmann T, Verma IM. A transmissible retrovirus exposing human hypoxanthine phosphoribosyltransferase (HPRT): gene transfer into cells obtained from humans deficient in HPRT. *Proc Natl Acad Sci USA* 1983; **80**: 4709.
85. Barabas G, Zumoff PJ. Overview of Lesch-Nyhan disease. *Matheny Bull (Spec edn)* 1993; **III**: 1.

Adenine phosphoribosyl-transferase deficiency

Introduction	498	Treatment	500
Clinical abnormalities	499	References	500
Genetics and pathogenesis	499		

MAJOR PHENOTYPIC EXPRESSION

Renal calculi and crystalluria, excretion of 2,8-dihydroxyadenine, and deficiency of adenine phosphoribosyltransferase.

INTRODUCTION

Deficiency of adenine phosphoribosyl-transferase (APRT) was first reported in 1976 by Simmonds and colleagues [1] and by Debray and colleagues [2], as a cause of urinary tract stones in children [1–3]. Initially, the calculi were mistaken for those of uric acid and this is still a potential problem [4] when a routine chemical colorimetric reaction for uric acid is used to determine the compound being excreted in excessive quantity, or if lucency of the stone is considered diagnostic. An effective therapeutic response may compound under diagnosis [5]. The disease has most often been recognized in children, in whom urinary calculi are rare and thus more likely to trigger a search for an unusual cause, but the disease is now recognized in adults more commonly than in children [4]. This is particularly true in Japan, where the disease is more commonly encountered [6].

The enzyme APRT (EC 2.4.2.7) ([Figure 65.1](#)) catalyzes the conversion of adenine to its mononucleotide (AMP). This enzyme and hypoxanthine guanine phosphoribosyl transferase (HPRT) ([Chapter 64](#)) have been referred to as purine salvage enzymes. Deficiency is readily documented by assay of erythrocyte lysates. The gene has been localized to chromosome 16q24.3 [7–9] and has been cloned and sequenced. Mutations have been identified [10, 11], including three mutations that account for 96 percent of the mutant alleles found in Japan [12].

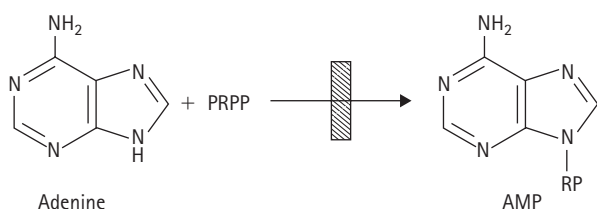


Figure 65.1 The reaction catalyzed by adenine phosphoribosyl-transferase (APRT). Magnesium is a cofactor. AMP, adenylic acid; PRPP, phosphoribosyl pyrophosphate.



Figure 65.2 An 18-month-old with adenine phosphoribosyl-transferase (APRT) deficiency who began passing stones at birth. At last report he was a young, fit 24-year-old. (Illustration was kindly provided by Dr H Anne Simmonds of the United Medical and Dental Schools, University of London.)

CLINICAL ABNORMALITIES

The clinical picture of APRT deficiency is entirely a function of the excretion of 2,8-dihydroxyadenine and its propensity to cause nephrolithiasis and nephropathy (Figure 65.2) [13–16]. The severity of the disease is quite variable, ranging from no symptoms to life-threatening renal disease. A number of asymptomatic patients with APRT deficiency has been reported; they were found because they were screened family members of a known patient [4, 13, 14, 17]. Onset of symptoms has ranged from birth to 74 years [5, 13]. Rarely, the disease may be indicated by the presence of brown spots on the diaper.

Patients may have hematuria, dysuria, crystalluria, or urinary tract calculi. The presenting complaint may be fever resulting from a complicating urinary tract infection. Calculi may also lead to renal colic or urinary retention. Obstructive crystals may lead to acute renal failure even in infancy. This may be reversible, but the long-term outcome in some patients is chronic renal failure leading to dialysis or renal transplantation [4, 15, 16, 18–22]. On the other hand, some patients are asymptomatic.

Plain roentgenograms of the abdomen are usually negative in these patients since 2,8-dihydroxyadenine stones, like those of uric acid, are radiolucent; rarely, admixture of calcium will render these stones radio-opaque. Abdominal ultrasound or intravenous urography should be done if there is a suspicion of radiolucent stones. Calculi are usually thought first to be those of uric acid and routine colorimetric analysis of the urine will not differentiate between uric acid and 2,8-dihydroxyadenine. A simple fluorescence method has been developed for the identification of 2,8-dihydroxyadenine stones. Calculi have been identified by a reddish brown appearance when wet, becoming gray when dry [23], of a round friable stone [17]. Mass spectrometry or scanning electron microscopy can be used for the correct identification of calculi [24].

It has become clear with increasing experience that there are no problems with other systems. Intelligence is normal. There is no abnormality of immune function.

GENETICS AND PATHOGENESIS

APRT deficiency is transmitted in an autosomal recessive fashion. Early reports of disease in heterozygotes have not held up. Consanguinity has been observed [3, 13, 25, 26]. The disease has been divided into two subtypes: in type I erythrocyte lysates have no activity and in type II there is residual activity. Now that it is possible to determine the mutation, these distinctions may become less useful, but homozygous type II patients have been found only in Japan; type I patients have been widely distributed, including in Japan [12, 27]. The frequency of a heterozygous allele for APRT deficiency has been given as 0.4–1.2 percent in various Caucasian populations [28–30], but the incidence

of diagnosed homozygotes is much lower than would be expected from these figures. This would be consistent with the asymptomatic nature of some patients described.

The enzyme (Figure 65.1) is a dimer with a subunit molecular weight of approximately 19 kDa and 179 amino acids [31]. 2,6-Diaminopurine (DAP) and 8-azaadenine are substrates for the enzyme, as are many adenine analogs that are toxic to cells after conversion to their nucleotides. These compounds are used in selective media [32]. Cells from either type I or type II patients will not grow in azaserine–alanosine–adenine (AAA) medium, and they are resistant to the adenine analogs 6-methylpurine and 2,6-diaminopurine. APRT is widely distributed in tissues and it is most commonly assayed in erythrocyte lysates, where normal activity is 16–32 nmol/hour per mg hemoglobin.

In patients with the classic type I disease, there is less than 1 percent of control activity and immunoreactive protein [33–35]. Patients with residual enzyme activity of 10–25 percent of control in the type II group have displayed a variety of different properties, such as sigmoidal kinetics [27, 36], reduced affinity for phosphoribosylpyrophosphate (PRPP) and altered heat stability. Decreased amounts of immunoprotein have been found [37].

Heterozygotes for type I deficiency have activity in hemolysates approximating 25 percent of control. Levels of immunoreactive protein may be 22 percent to normal [33–35]. In contrast, in lymphoblasts or fibroblasts, activity is 46 percent and cross-reacting material (CRM) 41 percent of control. This raises the issue of distinguishing type II homozygotes from type I heterozygotes. Testing for resistance of cultured cells to medium containing DAP or 6-methylpurine should resolve the question [28]. Documentation of the mutation is a better approach to these distinctions. In general, anyone with APRT activity of 25 percent or more will have no clinical symptoms.

The gene at 16q24 is the most telomeric gene on chromosome 16 [38]. It contains five exons and a 540 base pair coding region [39, 40]. The promoter region, like that of other housekeeping genes contains no TATA or CCAAT boxes, but contains 5GC boxes that are transcription factor binding sites [39]. A variety of mutations has been identified; many of them single base substitutions. Many patients are compounds of two mutant alleles. A T insertion in intron 4 at the splice donor site has been found in five families from Europe and the United States; it leads to abnormal splicing and loss of exon 4 in the mRNA [11, 14, 41–43]. The T insertion creates an *MseI* restriction site that is useful in diagnosis [42]. Another intron 4 splice donor site mutation, a G-to-T transversion, would also disrupt splicing. A relatively common mutation in Britain and Iceland was an A-to-T change in exon 3, which converts aspartate 65 to valine [11]. Five Icelandic patients were homozygous for this mutation.

Among Japanese patients, the most common mutation is a T-to-C mutation in exon 5 which changes methionine

136 to threonine (M136T) [12, 44]. This has to date been found exclusively in type II patients. Actually, three mutations account for 95 percent of the mutant alleles in Japanese patients [12]. The M136T accounts for 67 percent. The other two are a G-to-A substitution which changed tryptophan 98 to a stop codon in patients with the type I phenotype [45, 46], and a four base, CCGA insertion in exon 3. Restriction fragment length polymorphism in this area has been useful in family and population studies, leading, for instance, to a prediction that the M136T mutation has been in existence since at least 300BC [47]. This mutation has also been referred to as APRT*J [47]. A partial deficiency of APRT has also been reported in a patient with the type II mutation on one allele and a null or type I mutation on the other allele [48].

The direct consequence of deficiency of APRT is the accumulation of adenine, which is oxidized in the presence of xanthine oxidase to 2,8-dihydroxyadenine, which is very insoluble. The solubility of 2,8-dihydroxyadenine in water is less than 3 mg/L [13], but the compound may be supersaturated in urine. The excretion of adenine and 2,8-dihydroxyadenine occurs in a ratio of 1:1.5. 8-Hydroxyadenine is excreted in lesser amount. Concentrations of 2,8-dihydroxyadenine up to 80 mg/L (0.5 mmol/L) have been found in patients [13].

APRT deficiency has occurred in patients with Morquio syndrome [49]. The *GALNS* gene which is mutated in Morquio syndrome is located adjacent to APRT at 16q24.3 with APRT telomeric. A 100-kb deletion was found. A Japanese patient was found [50] with a submicroscopic deletion at *GALNS* and APRT on one allele and a point mutation (R386C) in APRT on the other.

TREATMENT

Therapy is aimed at reducing the formation of 2,8-dihydroxyadenine by the use of a low purine diet and allopurinol [3, 13, 15, 51, 52]. A dose of allopurinol of 10 mg/kg per day up to 300 mg in an adult has virtually eliminated 2,8-dihydroxyadenine from the urine [15, 53]. Adenine still accumulates. A high fluid intake is prudent. Alkali therapy is not beneficial; the solubility of 2,8-dihydroxyadenine is not altered by changes of urinary pH in the range obtainable physiologically. Stones already formed may be treated with lithotripsy [54–56].

REFERENCES

1. Simmonds HA, Van Acker KJ, Cameron JS, Snedden W. The identification of 28-dihydroxyadenine a new compound of urinary stones. *Biochem J* 1976; **157**: 485.
2. Debray H, Cartier P, Temstet A, Cendron J. Child's urinary lithiasis revealing a complete deficit in adenine phosphoribosyltransferase. *Pediatr Res* 1976; **10**: 762.
3. Barratt TM, Simmonds HA, Cameron JS *et al.* Complete deficiency of adenine phosphoribosyltransferase. A third case presenting as renal stones in a young child. *Arch Dis Child* 1979; **54**: 25.
4. Ceballos-Picot I, Perignon JL, Hamet M *et al.* 28-Dihydroxyadenine urolithiasis an underdiagnosed disease. *Lancet* 1992; **339**: 1050.
5. Simmonds HA, Van Acker KJ, Sahota AS. 2, 8-dihydroxyadenine urolithiasis. *Lancet* 1992; **339**: 1295.
6. Kamatani N, Terai C, Kuroshima S *et al.* Genetic and clinical studies on 19 families with adenine phosphoribosyltransferase deficiencies. *Hum Genet* 1987; **75**: 163.
7. Kahan B, Held KR, DeMars R. The locus for human adenine phosphoribosyltransferase on chromosome No 16. *Genetics* 1974; **78**: 1143.
8. Tischfield JA, Ruddle FH. Assignment of the gene for adenine phosphoribosyltransferase to human chromosome 16 by mouse-human somatic cell hybridization. *Proc Natl Acad Sci USA* 1974; **71**: 45.
9. Fratini A, Simmers RN, Callen DF *et al.* A new location for the human adenine phosphoribosyltransferase gene (APRT) distal to the haptoglobin (HP) and fra (16)(q23)(FRA16D) loci. *Cytogenet Cell Genet* 1986; **43**: 10.
10. Sahota A, Chen J, Stambrook PJ, Tischfield JA. Mutational basis of adenine phosphoribosyltransferase deficiency. *Adv Exp Med Biol* 1991; **309B**: 73.
11. Sahota A, Chen J, Stambrook PJ, Tischfield JA. Genetic basis of adenine phosphoribosyltransferase deficiency. In: Gresser U (ed.) *Molecular Genetics, Biochemistry and Clinical Aspects of Disorders of Purine and Pyrimidine Metabolism*. Heidelberg: Springer-Verlag, 1993: 579.
12. Kamatani N, Hakoda M, Otsuka S *et al.* Only three mutations account for almost all defective alleles causing adenine phosphoribosyltransferase deficiency in Japanese patients. *J Clin Invest* 1992; **90**: 130.
13. Sahota AS, Tischfield JA, Kametani N, Simmonds AH. Adenine phosphoribosyltransferase deficiency and 28-dihydroxyadenine lithiasis. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic and Molecular Bases of Inherited Disease*, 8th edn. New York: McGraw Hill, 2001: 2571–84.
14. Van Acker KJ, Simmonds HA, Potter CF, Cameron JS. Complete deficiency of adenine phosphoribosyltransferase: report of a family. *N Engl J Med* 1977; **297**: 127.
15. Greenwood MC, Dillon MJ, Simmonds HA *et al.* Renal failure due to 28-dihydroxyadenine urolithiasis. *Eur J Pediatr* 1982; **138**: 346.
16. Fye KH, Sahota A, Hancock DC *et al.* Adenine phosphoribosyltransferase deficiency with renal deposition of 28-dihydroxyadenine leading to nephrolithiasis and chronic renal failure. *Arch Intern Med* 1993; **153**: 767.
17. Laxdal T, Jonasson TA. Adenine phospho-ribosyltransferase deficiency in Iceland. *Acta Med Scand* 1988; **224**: 621.
18. Schabel F, Doppler W, Hirsch-Kauffman M *et al.* Hereditary deficiency of adenine phosphoribosyltransferase. *Paediatr Paedol* 1980; **15**: 233.
19. Gliklich D, Gruber HE, Matas AJ *et al.* 28-dihydroxyadenine lithiasis: report of a case first diagnosed after renal transplant. *Q J Med* 1988; **69**: 785.

20. Takemoto M, Nagano S. Urolithiasis containing 28-dihydroxyadenine: report of a case. *Acta Urol Jpn* 1979; **25**: 265.
21. Gagne ER, Deland E, Daudon M *et al*. Chronic renal failure secondary to 28-dihydroxyadenine deposition: the first report of recurrence in a kidney transplant. *Am J Kid Dis* 1994; **24**: 104.
22. de Jong DJ, Assmann KJ, De Abreu RA *et al*. 28-Dihydroxyadenine stone formation in a renal transplant recipient due to adenine phosphoribosyltransferase deficiency. *J Urol* 1996; **156**: 1754.
23. Ward ID, Addison GM. 2, 8-Dihydroxyadenine urolithiasis. *Lancet* 1992; **339**: 1296.
24. Winter P, Hesse A, Klocke K, Schaefer RM. Scanning electron microscopy of 28-dihydroxyadenine crystals and stones. *Scanning Microsc* 1993; **7**: 1075.
25. Kuroda M, Miki T, Kiyohara H *et al*. Urolithiasis composed of 28-dihydroxyadenine due to partial deficiency of adenine phosphoribosyltransferase. *Jpn J Urol* 1980; **71**: 283.
26. Ishidate T, Igarashi S, Kamatani N. Pseudodominant transmission of an autosomal recessive disease adenine phosphoribosyltransferase deficiency. *J Pediatr* 1991; **118**: 90.
27. Fujimori S, Akaoka I, Sakamoto K *et al*. Common characteristics of mutant adenine phosphoribosyltransferase. From four separate Japanese families with 28-dihydroxy-adenine urolithiasis associated with partial enzyme deficiencies. *Hum Genet* 1985; **7**: 171.
28. Hakoda M, Yamanaka H, Kamatani N, Kamatani N. Diagnosis of heterozygote states for adenine phosphoribosyltransferase. Deficiency based on detection of *in vivo* somatic mutants in blood T cells: application to screening of heterozygotes. *Am J Hum Genet* 1991; **48**: 552.
29. Simmonds HA. 28-dihydroxyadenine lithiasis. *Clin Chim Acta* 1986; **160**: 103.
30. Fox IH, La Croix S, Planet G, Moore M. Partial deficiency of adenine phosphoribosyltransferase in man. *Medicine* 1977; **56**: 515.
31. Wilson JM, O'Toole TE, Argos P *et al*. Human adenine phosphoribosyltransferase: complete amino acid sequence of the erythrocyte enzyme. *J Biol Chem* 1986; **261**: 13677.
32. Steglich C, DeMars R. Mutations causing deficiency of APRT in fibroblasts cultured from human heterozygous for mutant APRT alleles. *Somat Cell Genet* 1982; **8**: 115.
33. Kishi T, Kidani K, Komazawa Y *et al*. Complete deficiency of adenine phosphoribosyl-transferase: a report of three cases and immunologic and phagocytic investigations. *Pediatr Res* 1984; **18**: 30.
34. Wilson JM, Daddona PE, Simmonds HA *et al*. Human adenine phosphoribosyltransferase: immunochemical quantitation and protein blot analysis of mutant forms of the enzyme. *J Biol Chem* 1982; **257**: 1508.
35. O'Toole TE, Wilson JM, Gault MH, Kelley WN. Human adenine phosphoribosyltransferase: characterization from subjects with a deficiency of enzyme activity. *Biochem Genet* 1983; **21**: 1121.
36. Fujimori S, Akaoka I, Takeuchi F *et al*. Altered kinetic properties of a mutant adenine phosphoribosyltransferase. *Metabolism* 1986; **35**: 187.
37. Abe S, Hayasaka K, Narisawa K *et al*. Partial and complete adenine phosphoribosyltransferase deficiency associated with 28-dihydroxyadenine urolithiasis: kinetic and immunochemical properties of APRT. *Enzyme* 1987; **37**: 182.
38. Richards RI, Holman K, Lane S *et al*. Chromosome 16 physical map: mapping of somatic cell hybrids using multiplex PCR deletion analysis of sequence tagged sites. *Genomics* 1991; **10**: 1047.
39. Broderick TP, Schaff DA, Bertino AM *et al*. Comparative anatomy of the human APRT gene and enzyme: nucleotide sequence divergence and conservation of a non-random CpG dinucleotide arrangement. *Proc Natl Acad Sci USA* 1987; **84**: 3349.
40. Hidaka Y, Tarle SA, Kelley WN, Palella TD. Nucleotide sequence of the human APRT gene. *Nucleic Acids Res* 1987; **15**: 9086.
41. Hidaka Y, Palella TD, O'Toole TE *et al*. Human adenine phosphoribosyltransferase. Identification of allelic mutations at the nucleotide level as a cause of complete deficiency of the enzyme. *J Clin Invest* 1987; **80**: 1409.
42. Gathof BS, Zollner N. The restriction enzyme *MseI* applied for the detection of a possibly common mutation of the *APRT* locus. *Clin Invest* 1992; **70**: 535.
43. Chan J, Sahota A, Martin GF *et al*. Analysis of germline and *in vivo* somatic mutations in the human adenine phosphoribosyltransferase genes: mutational hotspots at the intron 4 splice donor site and at codon 87. *Mutat Res* 1993; **287**: 217.
44. Hidaka Y, Tarle SA, Fujimori S *et al*. Human adenine phosphoribosyltransferase deficiency. Demonstration of a single mutant allele common to the Japanese. *J Clin Invest* 1988; **81**: 945.
45. Sahota A, Chen J, Asako K *et al*. Identification of a common nonsense mutation in Japanese patients with type I adenine phospho-ribosyltransferase deficiency. *Nucleic Acids Res* 1990; **18**: 5915.
46. Mimori A, Hidaka Y, Wu VC *et al*. A mutant allele common to the type I adenine phosphoribosyltransferase deficiency in Japanese subjects. *Am J Hum Genet* 1991; **48**: 102.
47. Kamatani N, Teral C, Kim SY *et al*. The origin of the most common mutation of adenine phosphoribosyltransferase among Japanese goes back to a prehistoric era. *Hum Genet* 1996; **98**: 596.
48. Takeuchi H, Kaneko Y, Fujita J, Yoshida O. A case of compound heterozygote for adenine phosphoribosyltransferase deficiency (APRT*J/APRT*QO) leading to 28-dihydroxyadenine urolithiasis: review of the reported cases with 28-dihydroxyadenine stones in Japan. *J Urol* 1993; **149**: 824.
49. Wang L, Ou X, Sebesta I *et al*. Combined adenine phosphoribosyltransferase and N-acetylgalactosamine-6-sulfate sulfatase deficiency. *Mol Genet Metab* 1999; **68**: 78.
50. Fukuda S, Tomatsu S, Masuno M *et al*. Mucopolysaccharidosis IVA: submicroscopic deletion of 16q24.3 and a novel R386C mutation of N-acetylgalactosamine-6-sulfate sulfatase gene in a classical Morquio disease. *Hum Mutat* 1996; **7**: 123.
51. Cartier P, Hamet M, Vincens A, Perignon JL. Complete adenine phosphoribosyl-transferase (APRT) deficiency in two siblings: report of a new case. *Adv Exp Biol Med* 1980; **122A**: 343.

52. Chevet D, Le Pogamp P, Gie S *et al.* 28-Dihydroxy-adenine (28-DHA) urolithiasis in an adult – complete adenine phosphoribosyl-transferase deficiency – family study. *Kidney Int* 1984; **26**: 226.
53. Simmonds HA, Cameron JS, Barratt TM *et al.* Purine enzyme defects as a cause of acute renal failure in childhood. *Pediatr Nephrol* 1989; **3**: 433.
54. Jung P, Becht E, Ziegler M *et al.* New diagnostic and therapeutic aspects of 28-dihydroxyadenine lithiasis. Another case of complete adenine phosphoribosyltransferase deficiency. *Eur J Urol* 1988; **14**: 493.
55. Coupris L, Champion G, Duverne C *et al.* La lithiase 28-dihydroxyadeninique 2 nouvelles observations pédiatriques d'un déficit métabolique méconnu. Apport de la lithotrypsie extra-corporelle. *Chir Pediatr* 1990; **31**: 26.
56. Frick J, Sarica K, Kohle R, Kunit G. Long-term follow-up after extracorporeal shock wave lithotripsy in children. *Eur Urol* 1991; **19**: 225.

Phosphoribosylpyrophosphate synthetase and its abnormalities

Introduction	503	Treatment	505
Clinical abnormalities	504	References	505
Genetics and pathogenesis	504		

MAJOR PHENOTYPIC EXPRESSION

Hyperuricemia, uricosuria, hematuria, crystalluria, urinary tract calculi, gouty arthritis, nephropathy, sensorineural deafness, and abnormal phosphoribosylpyrophosphate synthetase, in which activity is greater than normal.

INTRODUCTION

Accelerated activity of phosphoribosylpyrophosphate (PRPP) synthetase associated with overproduction of uric acid and gout was first reported in 1972 by Sperling and colleagues [1, 2]. A small number of patients has now been identified, establishing the relationship with hyperuricemia [1–10]. In some kindreds, there has been sensorineural deafness [11–13].

5-Phosphoribosylpyrophosphate synthetase (EC 2.7.6.2) (Figure 66.1) catalyzes the initial step in the *de novo* synthesis of purines in which ribose-5-P reacts with adenosine triphosphate (ATP) to form PRPP. The PRPP

formed provides the substrate for the first rate-limiting step in the ten-step purine *de novo* pathway. Increased quantities of intracellular PRPP lead to overproduction of purine *de novo* and of uric acid. PRPP synthetase is coded for by two genes on the X chromosome at Xq22-24 and Xp22.2-22.3 [14, 15]. The genes have been cloned and sequenced [16] and referred to as *PRPS1* and *S2* [17]. A small number of point mutations has been defined in *PRPS1* in patients with overactivity and altered allosteric properties of the enzyme.

In six patients with overactivity of PRPP synthetase, no mutation in the cDNA of *PRPS1* or *S2* were found; instead, there were increased quantities of the S1 isoform whose physical and catalytic properties were normal [18].

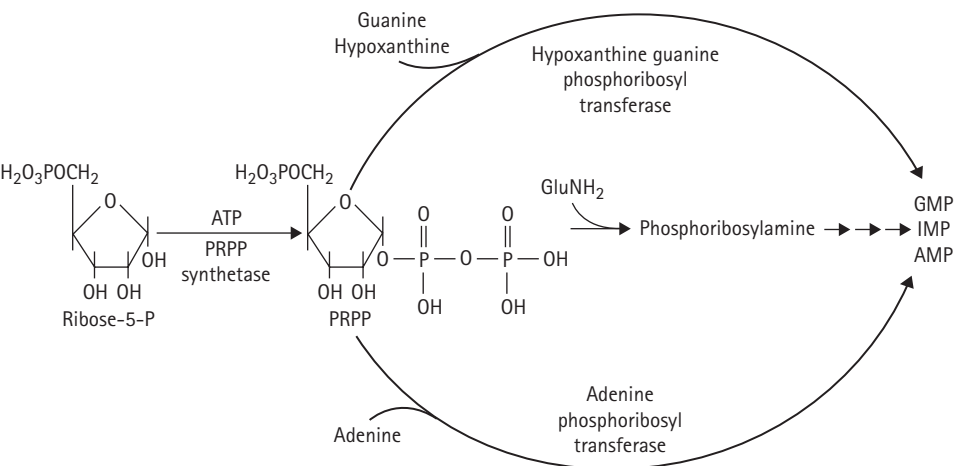


Figure 66.1
Phosphoribosylpyrophosphate (PRPP) synthetase. The role of the product PRPP is central in the interrelation of purines and their nucleotides.

CLINICAL ABNORMALITIES

The invariant clinical features of this disease are hyperuricemia and uricosuria. Therefore, a patient is subject to any of the clinical consequences of the accumulation of uric acid in body fluids. Gouty arthritis has been reported with onset as early as 21 years of age [1]. Renal colic has been observed, as well as the passage of calculi [3]. One boy developed hematuria at the age of two months and was found to have crystalluria, hyperuricemia, and uricosuria [4]. In families with this early onset phenotype, females have manifestations of hyperuricemia prior to menopause [13].

A small number of families has been reported [5, 6, 11, 12] in which deafness has been associated with an abnormally active PRPP synthetase. In one family, there were three involved males, each of whom also had severe neurodevelopmental impairment. The mother had high tone deafness. A large kindred had previously been reported in which there were X-linked deafness and hyperuricemia; an enzyme defect was not demonstrated at the time of report [19].

Another patient [5] appeared initially to have impaired mental development and his behavior was thought to be autistic (Figure 66.2). With time, it became apparent that he was deaf and his behavior was quite appropriate (Figure 66.3). As an infant, he failed to cry with tears and was found to have absent lachrymal glands. Other structural anomalies were aglandular hypospadias and hypoplastic teeth. The relationship of all of these problems to the

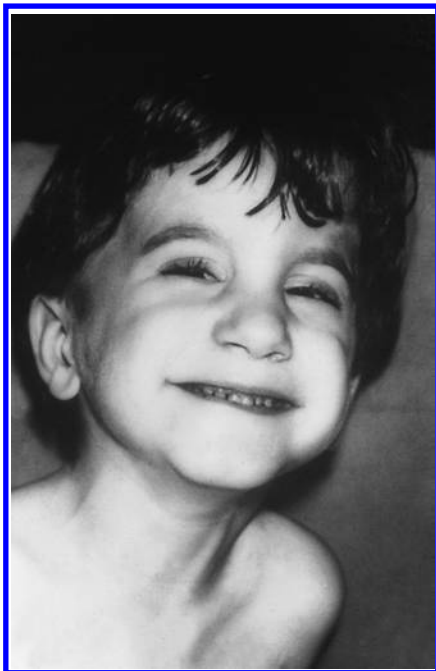


Figure 66.2 SM: A three-year-old with an abnormal phosphoribosylpyrophosphate synthetase. The odd grimace was characteristic. (Reprinted from the *Journal of Pediatrics* [5] with permission from Elsevier).

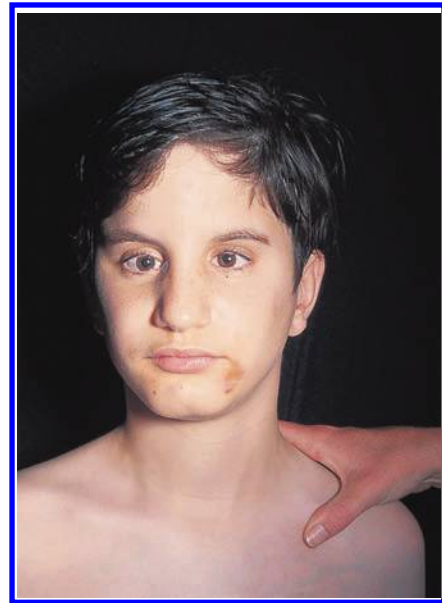


Figure 66.3 SM: At 14 years of age.

metabolic abnormality is not clear, but the mother also had the abnormal PRPP synthetase and hearing loss, and she and her father had problems with behavior.

Patients with late teenage or young adult gout or urolithiasis have been exclusively male and have had no neurologic abnormalities [10, 13]. These patients have had overabundance of the normal S1 isoform.

All of these patients have had increased amounts of uric acid in the blood and urine. In our patient, prior to treatment, the concentration in serum ranged from 8.5 to 11.6 mg/dL [5]. Urinary excretion ranged from 1.84 to 3.26 mg/mg creatinine. In the initial proband [1], uric acid excretion was 2400 mg per 24 hours. Overproduction of purine *de novo* was documented [5] by measuring the *in vivo* conversion of ^{14}C -glycine to urinary uric acid. In 7 days, 0.7 percent of the isotope of glycine administered was converted to uric acid – seven times the control level of 0.1 percent.

GENETICS AND PATHOGENESIS

The S1 isoform of PRPP synthetase, while coded for by a gene on the long arm of the X chromosome [14] may be fully recessive [1] or may be expressed in the heterozygous female [3]. This could reflect different degrees of Lyonization. On the other hand, it is easier for an overactive enzyme, rather than the more common deficient one encountered in inborn errors, to function as an X-linked dominant.

The *PRPS1* gene has been localized to Xq22-24 [15, 20]. The human PRPP *S1* gene spans 30 kb and has 17 exons; the cDNA encodes a transcript of 2.3 kb and a protein of 317 amino acids. The *PRPS2* gene is located at Xp22.3-22.2 [15, 21] on the other end of the chromosome. Regardless of

the location, the two genes have over 90 percent homology in the cDNA and amino acid sequence.

The *PRPS2* gene codes for a transcript in testis, a 318 amino acid sequence [22–24]. The *PRPS1* gene is located between the α -galactosidase (*GLA*) and *HPRT1* genes [14]. The *PRPS1* and two genes both undergo inactivation with Lyonization [25]. The *PRPS2* gene is situated between two genes on the short arm that escape inactivation distally and ZFX apromoximally. The *XIST* gene is transcribed only from the inactive X chromosome. A third gene (*PRPS3*) maps to chromosome 7 and is expressed only in testis [22].

In six male patients with overactivity of PRPP synthetase and resistance to purine nucleotide feedback, there was a single base transition, which led to a single amino acid change at positions 51 (D51H), 89, 113 (N113S), 128 (L128I), 182 (D182H), and 192 (H192Q) [16, 17].

The molecular basis of the disease in the patients with superactive enzyme activity is an altered PRPP synthetase structure. Activity may be three times that of the normal enzyme [3]. In one of the families studied, increased enzyme activity was demonstrable only at low concentrations of phosphate, and there was diminished responsiveness to feedback inhibition by purine nucleotides [2]. In another family, an elevated level of enzyme-specific activity was demonstrable over a wide range of phosphate concentrations, and feedback inhibition was normal [3]. The amounts of immunoreactive enzyme protein were normal [26]. These observations indicate the presence in normal amounts of a protein in which structural alteration leads to increased specific activity. The data are consistent with the presence of two important sites on the enzyme: a catalytic site altered by one mutation and a regulatory site altered by the other. In one patient, the altered structure affected both catalytic and regulatory sites [6]. The enzyme may have increased affinity for the substrate ribose-5-phosphate [27].

In addition to its other properties, the PRPP synthetase in one patient had diminished stability to heat [6]. Distinctly diminished levels were found in old as opposed to young erythrocyte obtained by density separation. For these reasons, the activity of PRPP as determined in erythrocyte lysates was not elevated, but in fact was lower than normal. This same observation was made in another family in which erythrocyte levels of PRPP were not elevated [11]. In the first patient, the activity of PRPP synthetase in hemolysates was less than 10 percent of normal at concentrations of inorganic phosphate over 1 mM. In this sense, a direct assay that indicates a deficiency of the erythrocyte's enzyme could be the clue to the presence of an abnormal enzyme that is superactive *in vivo*. Nevertheless, in a patient who appears clinically to be a candidate for a diagnosis of PRPP synthetase overactivity, a normal result of an erythrocyte assay should be followed up by an intact cell method.

Intact cultured fibroblasts can be shown to incorporate each of the purine bases, adenine, guanine, and hypoxanthine, more rapidly into nucleotides than do controls [6]. Adenosine conversion to nucleotide is normal.

Incorporation of ^{14}C -formate into formylglycinamide ribotide (FGAR) in the presence of azaserine is also accelerated. These findings indicate the presence of increased intracellular concentrations of PRPP. Concentrations can be measured in fibroblasts or lymphoblasts and found to be elevated [6].

The single amino acid substitutions in the S1 enzyme are clearly scattered over much of the protein; yet they all lead to decreased responsiveness to feedback inhibition by adenosine diphosphate (ADP) and guanosine diphosphate (GDP) [16]. Also the binding of MgATP to the active site is normal. The mechanism for the allosteric changes resulting in superactivity is unknown, but it must be structurally diffuse. Mechanisms for the increased transcription in the patients in whom no structural changes were found are even less clear, although a number of possibilities have been excluded [28].

In males with X-linked recessive Charcot-Marie-Tooth disease, Kim *et al.* [29] found mutations in *PRPS1* in which there was decreased enzyme activity. These patients had sensorineural deafness and the gene is known to be expressed in the cochlea. None of the patients had hyperuricemia. Similarly in Arts syndrome, an X-linked impaired mental development with early onset hypotonia, ataxia, and optic atrophy, loss of function mutations in *PRPS1* led to hypouricemia [30]. Missense mutations in this gene were also found in patients with X-linked nonsyndromic deafness [31].

TREATMENT

Allopurinol is the treatment of choice in overproduction of hyperuricemia. Treatment of abnormalities in PRPP synthetase is simpler than that of hypoxanthine guanine phosphoribosyl transferase (HPRT) deficiency, because in the presence of normal HPRT activity there is extensive reutilization of hypoxanthine accumulating behind the block in xanthine oxidase, leading to a substantial decrease in the overall excretion of oxypurines in the urine. In contrast, in HPRT deficiency, there is simple substitution of hypoxanthine or xanthine for uric acid, and the total oxypurine excretion does not change.

Hearing should be tested promptly and appropriate intervention provided.

REFERENCES

1. Sperling O, Eilma G, Persky-Brosh S, DeVries A. Accelerated erythrocyte 5-phosphoribosyl-1-pyrophosphate synthesis: a familial abnormality associated with excessive uric acid production and gout. *Biochem Med* 1972; **6**: 310.
2. Sperling O, Boer P, Persky-Brosh S *et al.* Altered kinetic property of erythrocyte phosphoribosylpyrophosphate synthetase in excessive purine production. *Eur J Clin Biol Res* 1972; **17**: 73.

3. Becker MA, Meyer LJ, Seegmiller JE. Gout with purine overproduction due to increased phosphoribosylpyrophosphate synthetase activity. *Am J Med* 1973; **55**: 232.
4. DeVries A, Sperling O. Familial gouty malignant uric acid lithiasis due to mutant phosphoribosyltransferase synthetase. *Urologie A* 1973; **12**: 153.
5. Nyhan WL, James JA, Teberg AJ *et al*. A new disorder of purine metabolism with behavioral manifestations. *J Pediatr* 1969; **74**: 20.
6. Becker MA, Raivio KO, Bakay B *et al*. Variant human phosphoribosylpyrophosphate synthetase altered in regulatory and catalytic functions. *J Clin Invest* 1980; **65**: 109.
7. Nishida Y, Akaoka I, Horiuchi Y. Altered isoelectric property of a superactive 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase in a patient with clinical gout. *Biomed Med* 1981; **26**: 387.
8. Akaoka I, Fujimori S, Kamatani N *et al*. A gouty family with increased phosphoribosylpyrophosphate synthetase activity: case reports familial studies and kinetic studies of the abnormal enzyme. *J Rheumatol* 1981; **8**: 563.
9. García-Pavía P, Torres RJ, Rivero M *et al*. Phosphoribosylpyrophosphate synthetase overactivity as a cause of uric acid overproduction in a young woman. *Arthritis Rheum* 2003; **48**: 2036.
10. Becker MA, Losman MH, Rosenberg AL *et al*. Phosphoribosylpyrophosphate synthetase superactivity. A study of five patients with catalytic defects in the enzyme. *Arthritis Rheum* 1986; **29**: 880.
11. Simmonds HA, Webster DR, Wilson J *et al*. Evidence of a new syndrome involving hereditary uric acid overproduction neurological complications and deafness. In: DeBruyn CHMM, Simmonds HA, Muller MM (eds). *Purine Metabolism in Man*, IV. New York: Plenum Press, 1984: 97.
12. Simmonds HA, Webster DR, Lingam S, Wilson J. An inborn error of purine metabolism deafness and neurodevelopmental abnormality. *Neuropediatrics* 1985; **16**: 106.
13. Becker MA, Puig JG, Mateos FA *et al*. Inherited superactivity of phosphoribosylpyrophosphate synthetase: association of uric acid overproduction and sensorineural deafness. *Am J Med* 1988; **85**: 383.
14. Becker MA, Yen RCK, Itkin P. Regional localization of the gene for human phosphoribosylpyrophosphate synthetase on the X chromosome. *Science* 1979; **203**: 1016.
15. Becker MA, Heidler SA, Bell GI *et al*. Cloning of cDNAs for human phosphoribosylpyrophosphate synthetases 1 and 2 and X chromosome localization of PRPS1 and PRPS2 genes. *Genomics* 1990; **8**: 555.
16. Becker MA, Smith PR, Taylor W *et al*. The genetic and functional basis of purine nucleotide feedback-resistant phosphoribosylpyrophosphate synthetase superactivity. *J Clin Invest* 1995; **96**: 2133.
17. Roessler BJ, Nosal JM, Smith PR *et al*. Human X-linked phosphoribosylpyrophosphate synthetase superactivity is associated with distinct point mutations in the PRPS1 gene. *J Biol Chem* 1993; **268**: 26476.
18. Becker MA, Taylor W, Smith PR, Ahmed M. Overexpression of the normal phosphoribosylpyrophosphate synthetase isoform 1 underlies catalytic superactivity of human phosphoribosylpyrophosphate synthetase. *J Biol Chem* 1996; **271**: 19894.
19. Rosenberg AL, Bergstrom L, Troost BT, Bartholomew BA. Hyperuricaemia and neurological deficits: a family study. *N Engl J Med* 1970; **282**: 992.
20. Becker MA, Yen RCK, Itkin P *et al*. Regional localization of the gene for human phosphoribosylpyrophosphate synthetase on the X-chromosome. *Science* 1979; **203**: 1016.
21. Taira M, Kudoh J, Minoshima S *et al*. Localization of human phosphoribosylpyrophosphate synthetase subunit I and II genes (PRPS1 and PRP S2) to different regions of the chromosome and assignment of two PRPS1-related genes to autosomes. *Somat Cell Mol Genet* 1989; **15**: 29.
22. Taira M, Iizasa T, Shimada H *et al*. A human testis-specific mRNA for phosphoribosylpyrophosphate synthetase that initiates from a non-AUG codon. *J Biol Chem* 1990; **265**: 16491.
23. Taira M, Iizasa T, Yamada K *et al*. Tissue-differential expression of two distinct genes for phosphoribosyl pyrophosphate synthetase and existence of the testis. *Biochim Biophys Acta* 1989; **1007**: 203.
24. Iizasa T, Taira M, Shimada H, Tatibana M. Molecular cloning and sequencing of human cDNA for phosphoribosylpyrophosphate synthetase subunit II. *FEBS Lett* 1989; **13**: 47.
25. Wang JC, Passage MB, Ellison J *et al*. Physical mapping of loci in the distal half of the short arm of the human X chromosome: implications of the spreading of inactivation. *Somat Cell Mol Genet* 1992; **18**: 195.
26. Becker MA, Kostel PJ, Meyer LJ, Seegmiller JE. Human phospho-ribosylpyrophosphate synthetase: increased enzyme specific activity in a family with gout and excessive purine synthesis. *Proc Natl Acad Sci USA* 1973; **70**: 2749.
27. Becker MA, Losman MH, Simmonds HA. Inherited phosphoribosyl-pyrophosphate synthetase superactivity due to aberrant inhibitor and activator responsiveness. In: Nyhan WL, Thompson LF, Watts RWE (eds). *Purine and Pyrimidine Metabolism in Man, V. Part A: Clinical Aspects Including Molecular Genetics*. New York: Plenum Press, 1986: 59.
28. Ahmed M, Taylor W, Smith PR, Becker MA. Accelerated transcription of PRPS1 in X-linked overactivity of normal human phosphoribosylpyrophosphate synthetase. *J Biol Chem* 1999; **274**: 7482.
29. Kim HJ, Sohn KM, Shy ME *et al*. Mutations in PRPS1, which encodes the phosphoribosylpyrophosphate synthetase enzyme critical for nucleotide biosynthesis peripheral neuropathy with hearing loss and optic neuropathy (CMT5X). *Am J Hum Genet* 2007; **81**: 552.
30. De Brouwer APM, Williams KL, Duley JA *et al*. Arts syndrome is caused by loss-of-function mutations in PRPS1. *Am J Hum Genet* 2007; **81**: 507.
31. Liu X, Han D, Li J *et al*. Loss-of-function mutations in the PRPS1 gene cause a type of nonsyndromic X-linked sensorineural deafness, DFN2. *Am J Hum Genet* 2010; **86**: 65.

Adenosine deaminase deficiency

Introduction	507	Treatment	510
Clinical abnormalities	507	References	511
Genetics and pathogenesis	509		

MAJOR PHENOTYPIC EXPRESSION

Severe combined immunodeficiency disease, involving immunoglobulins and cell-mediated immunity; clinical immunodeficiency triad of persistent diarrhea, progressive pulmonary disease, and extensive moniliasis; skeletal abnormalities; and deficiency of adenosine deaminase.

INTRODUCTION

The discovery of the association between adenosine deaminase (ADA) (EC 3.5.4.4) deficiency and severe combined immunodeficiency disease in the early 1970s [1] provided exciting evidence of metabolic causation of immunodeficiency. This established a relationship between the metabolism of purines and developmental immunobiology and this was reinforced by the discovery of purine nucleoside phosphorylase deficiency.

The discovery of this disorder was an interesting example of important observations made by a prepared mind. Giblett, a pediatric pathologist [1, 2], was reviewing polymorphic markers in candidates for bone marrow transplantation and found one deficient in ADA; a sample from a second unrelated patient studied a week later was also ADA-deficient. Within a year, a number of immunodeficient patients with ADA deficiency were reported [3]. Giblett and colleagues [4] used the same approach to discover purine nucleoside phosphorylase (PNP) (EC 2.4.2.1) deficiency.

Adenosine deaminase and PNP deficiencies represent enzymatic defects in the metabolism of purines that affect primarily cells of the immune system. The mechanism appears to be the accumulation of purine nucleotides which are toxic to T and B cells [5]. Adenosine deaminase is an enzyme of purine interrelations, which converts adenosine to inosine (Figure 67.1). This is an important reaction because adenosine is not a substrate for nucleoside phosphorylase, which converts inosine and guanosine to

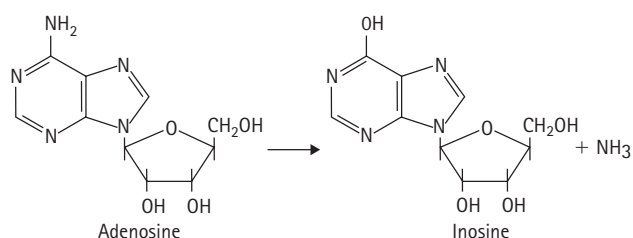


Figure 67.1 The adenosine deaminase reaction.

hypoxanthine and guanine. Adenosine deaminase is widely distributed in animal tissues. This enzyme is determined by a gene on chromosome 20q13.11 [6]. The gene for ADA has been cloned and sequenced [7]. A considerable number of mutations have been identified [8–10], most of them single amino acid changes.

CLINICAL ABNORMALITIES

Classic patients with ADA deficiency have a distinct syndrome of severe combined immunodeficiency disease (SCID). In common with other patients with SCID, they have both defective immunoglobulins or bone marrow-derived B-cell function and defective cell-mediated immunity or thymus-derived T-cell function. Patients with B-cell or humoral immunodeficiency have infections caused by organisms such as the pneumococcus with capsules, as well as some viruses. Patients with T-cell or

cell-mediated immunodeficiency have infections caused by opportunistic organisms, such as monilia, or by viruses. Patients with combined immunodeficiency have both types of infection.

Most patients with SCID present in the first months of life with failure to thrive and recurrent or persistent diarrhea [1, 3, 11–14]. The diagnosis is usually not made until the onset of infection that follows the disappearance of maternal antibody. Severe bacterial and viral infections occur very early in life. A majority of patients have had extensive candidiasis. Many have presented in the first weeks or months of life with thrush, diarrhea, and pneumonia. Many have had bacterial infections of the skin. Recurrent otitis media is common and pneumonia is a frequent complication. Many patients have died, often of infections with opportunistic organisms, some viral and some bacterial, that are not usually productive of severe infections in ordinary individuals.

Isolated defects of the cellular immune system, as well as severe forms of combined immunodeficiency, have been described in 10–15 percent of individuals with ADA deficiency [6, 15]. These patients have had a milder course of disease, often with a later onset. A number of patients has been observed with milder forms of the disease reflecting higher levels of residual ADA activity. Adult-onset immunodeficiency was reported in two sisters with a residual ADA activity of 5–13 percent [16]. Furthermore, ADA deficiency has been described without abnormality in immune function [17]. A tandem mass spectrometry (MS/MS) method for the detection of ADA deficiency has been developed [18]; so neonatal screening is now possible, and this should lead to complete ascertainment and better definition of the clinical spectrum. In general, it appears that immune function may be normal if levels of ADA are over 5 percent of control.

Patients with ADA deficiency, like those with other types of SCID, are at risk for the development of graft-versus-host disease if they receive blood transfusion from a donor with immunocompetent T cells. They are also at risk for disseminated diseases following immunization with live attenuated vaccines, such as poliomyelitis. Active infection has occurred following administration of oral polio vaccine. Known patients are better immunized for this disease with Salk inactivated vaccine.

The majority of patients reported with ADA deficiency have been infants with SCID and recurrent infections [19, 20]. Pneumonia may be caused by *Pneumocystis carinii* or by viruses. Candidiasis may involve the skin, the mucosa of the mouth, esophagus, or vagina, and stool cultures may reveal this organism. Patients surviving infancy may have pulmonary insufficiency, a consequence of repeated infection. Among late onset presentations were pulmonary insufficiency and recurrent warts in adult sisters [21].

Autoimmune disease is also a feature of ADA deficiency. Presentations have included autoimmune thyroid insufficiency [22, 23], autoimmune thrombocytopenia [24], and fatal autoimmune hemolytic anemia [25].

Neurologic abnormalities may occur in ADA deficiency; they are more common in PNP deficiency. They may be a consequence of infection or possibly of the severe failure to thrive. However, in some patients, neurologic abnormality has appeared to be a metabolic consequence of the disease. Patients have had spasticity, nystagmus, tremors, dystonic posturing, athetosis, hypotonia, and head lag [20, 26–28]. In at least one patient, improvement was documented after successful treatment with enzyme replacement [26]. Developmental delay has been reported to be more prevalent in ADA-deficient patients with SCID than in ADA-normal patients with SCID [20]. In a similar study of patients with SCID treated with bone marrow transplantation [29], tests of cognitive function revealed no differences between the two groups, ADA-deficient and non-ADA-deficient. However, among the ADA-deficient patients, there was significant inverse correlation between the levels of deoxyadenosinetriphosphate (dATP) at diagnosis and IQ. In behavioural assessment, the ADA-SCID patients functioned in the pathologic range in all domains, while the non-ADA patients scored in the normal range. Neurologic examinations were unremarkable in these patients and none had seizures.

Among neurologic complications, a high incidence of bilateral sensorineural deafness has been observed [29].

In a report of 12 patients with ADA deficiency successfully treated with bone marrow transplantation, 58 percent had bilateral deafness [30].

Hepatic dysfunction has been observed in this disease [31, 32] and many patients have elevated serum transaminase levels; levels have improved with replacement therapy. Recurrent hepatitis has been followed by chronic hepatobiliary disease. B-cell lymphomas have been related to infection with Epstein–Barr virus.

Physical examination may be remarkable only for evidence of infection, poor growth, and failure to thrive. Absence of lymphatic tissue may be striking and this may be evident on palpation or examination of the pharynx. It is often first recognized roentgenographically. In roentgenograms, the upper mediastinum is narrow. In lateral views, there may be retrosternal radiolucency and no thymus shadow can be seen. Examination of the blood reveals profound lymphopenia [32]. Total lymphocyte count may be less than 500 per mL. Most of these patients have chronic pulmonary changes such as those seen in infections with *Pneumocystis carinii*.

A bony dysplasia is an impressive feature of the syndrome [33]. The sacroiliac notch may be large, as in achondroplasia and the ilium flares outward, resembling Mickey Mouse ears. The acetabular angle is reduced, also like that of achondroplasia. The pubis is short and the ischium squared off. The ribs are flared, enlarged anteriorly, and cupped at the costochondral ends, resembling changes seen in rickets. In the spine, there is platyspondyly and an appearance reminiscent of mucopolysaccharidosis. In contrast to the spine in achondroplasia, the interpedicular distance in these patients does not decrease from L1

to L5. Growth arrest lines may be unusually thick. Roentgenograms of the bones may reveal profound osteoporosis. One patient had compression fractures of two vertebral bodies.

Adult onset disease is increasingly being recognized [34] with recurrent infections with opportunistic organisms, such as oral and vaginal candidiasis or viral warts, as well as commonly pathogenic organisms, causing for instance tuberculosis. A patient was reported with Burkitt lymphoma following successful treatment with polyethylene glycol-linked ADA (PEG-ADA) [35].

Skin tests for delayed hypersensitivity are deficient and skin tests for candida are negative in patients known to have had candidal infection. Skin tests for streptokinase and other antigens are negative. The response of lymphocytes *in vitro* to phytohemagglutinin and other lectins is reduced or absent, and the formation of T-cell rosettes is poor. All of the immunoglobulins in most of these patients are decreased in concentration, once the infant is old enough to have lost immunoglobulin transferred from the mother. These include IgG, IgM, IgA, and others, but often it is to a variable degree of each. The antibody response to the injection of an immunizing antigen is faulty.

Pathologic examination of the thymus at autopsy has revealed a very small organ with little differentiation into lobules. No Hassall's corpuscles were seen. There was no central medullary area and no differentiation into cortex and medulla. Huber and Kersey [36], who analyzed tissues from four patients with adenosine deaminase deficiency and five without, all of whom had died of combined immunodeficiency disease, believed that they could distinguish between the two groups. The patients without ADA deficiency appeared to have failed to develop thymic tissue in early embryonic life, whereas the ADA-deficient patients had what they called 'extreme involution'. The thymus in these patients appeared to have known better days.

GENETICS AND PATHOGENESIS

Adenosine deaminase deficiency is transmitted as an autosomal recessive disease. In heterozygous carriers for adenosine deaminase deficiency, levels of enzyme activity are about half the normal level [5, 37–39], but detection of carriers by enzyme assay in erythrocytes or fibroblasts is not reliable. Polymorphism in the ADA cDNA has been successfully used for this purpose, and of course in a family in which the mutation is known, this is a reliable approach to carrier detection. The incidence of the disease from neonatal screening in New York approximated one in 400,000–500,000 births [19, 40]. ADA deficiency accounts for 15 percent of the patients with SCID and one-third of patients with autosomal recessive SCID [41]. Prenatal diagnosis has been accomplished by assay of the enzyme in cultured amniocytes and chorionic villus samples [37, 42, 43].

The molecular basis of the disease is the deficiency of the activity of ADA, a 41-kDa single polypeptide chain enzyme, the N terminal of which is post-translationally removed to yield 332 amino acids [44]. The ADA gene [7] consists of 12 exons spanning about 32,000 bases of DNA. Analyses of the ADA genes isolated from patients with ADA deficiency have revealed a heterogeneous pattern of mutations as causes of deficient enzyme activity [8, 9, 45–48]. Most patients are compounds of two mutant genes. Severe combined immunodeficiency and low levels of mRNA and protein have been seen with a 5-bp deletion in exon 10 [45] and a glycine 20 to arginine point mutation, while later-onset, milder disease was found with a point mutation changing arginine 253 to proline [48] and substitution at 156 of histidine for arginine. Splicing and other missense mutations have been identified [46]. The same mutation found in more than one family has often arisen at CpG hot spots [49, 50]. A329V, a relatively common mutation has been found in a number of unrelated patients [8]; so has R211H. Most deletions are small, but may introduce stop signals. Splicing site mutations, such as a G to A change in IVS10, which inserts 100 amino acids, have been observed in patients with more indolent disease, suggesting that alternate splicing may provide useful amounts of the wild-type enzyme [51, 52]. In a patient with late onset SCID diagnosed at the age of 16 years, a homozygous mutation in intron 11 and an 11p deletion of adjacent base pairs suppressed aberrant splicing, and T and B cells had 75 percent of normal ADA activity, and ADA protein of normal size indicating somatic reversion [10]. Prenatal diagnosis has been accomplished by the assessment of mutation in the ADA gene [53].

ADA catalyzes the irreversible deamination of adenosine (Figure 67.1) to form inosine. Deoxyadenosine also serves as a substrate for the enzyme. Intracellular adenosine is produced in the catabolism of RNA and also by the hydrolysis of S-adenosylhomocysteine, an intermediary in transmethylation reactions [54].

Adenosine deaminase may be assayed in the erythrocyte by means of a technique that measures ammonia liberated from adenosine. The enzyme in intact erythrocytes of normal individuals had a mean activity of 0.29 nmol/min per mL packed cells. There has been no detectable ADA activity in most patients studied [5, 38, 39]. A screening test has been developed [55] which permits the diagnosis on spots of dried blood on filter paper and is employed in neonatal screening.

In ADA deficiency, there is accumulation of adenosine and deoxyadenosine. Normal plasma concentrations of adenosine are 0.05–0.4 mM/L; levels of deoxyadenosine are below the level of detection. In ADA-deficient patients, plasma concentrations of adenosine and deoxyadenosine range from 0.5 to 10 mM/L. Large amounts of deoxyadenosine are excreted in the urine.

Inhibitors of ADA are toxic to cells. The pathophysiological mechanism by which ADA deficiency produces immunodeficiency appears to be the consequence of the

accumulation of adenosine and deoxyadenosine, which are converted to ATP and dATP. ATP and, especially, dATP have been shown to be toxic to lymphoid cells of the immune system [6, 56–60]. It is thought that accumulated dATP inhibits ribonucleotide reductase, which catalyzes the conversion of ribonucleotide diphosphates to deoxyribonucleotide diphosphates and in this way inhibits the synthesis of DNA [58]. Consistent with hypothesis, deoxynucleosides of cytosine, thymine, and guanine are capable of reversing the toxic effects of the adenosine deaminase inhibitor, deoxycytosine [59], but there appear to be many possible mechanisms of the deficiency of immune function. Deoxyadenosine itself also leads to chromosomal breakage through inhibition of DNA repair.

Adenosine deaminase inhibitors, coformycin and deoxycytosine, produce a metabolic pattern in normal cells similar to that of ADA-deficient cells, and so does an inhibitor of nucleoside transport; neither compound had any effect on ADA-deficient cells [59]. Lymphocytes and lymphoblasts undergo apoptosis when treated with deoxyadenosine [61]. The activity of S-adenosylhomocysteine (SAH) hydrolase is reduced in ADA deficiency, a consequence of suicide-like inactivation by deoxyadenosine [62]. Accumulation of adenosylhomocysteine could inhibit transmethylation.

Adenosine also serves as a regulator of blood flow and an inhibitor of platelet aggregation, lipolysis, and neurotransmitter release. It modulates beta-adrenergic receptor and insulin-mediated responses; it stimulates steroidogenesis and histamine release; and it inhibits superoxide and hydrogen peroxide release from neutrophils [63]. It is not clear that any of these functions is altered in ADA deficiency.

Newborn screening for this disease, and other causes of SCID, has been developed in dried blood spots [64] and incorporated into state programs for newborns. The

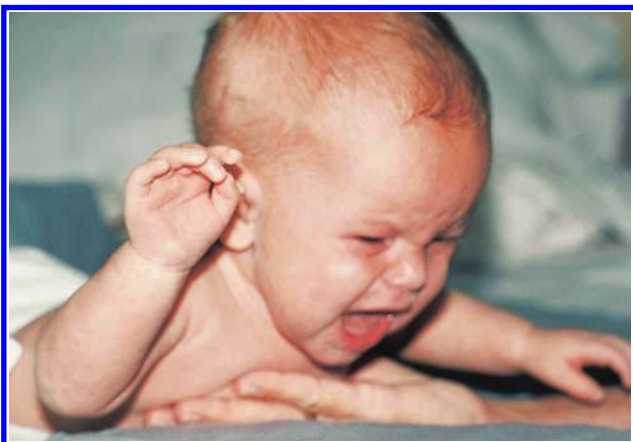


Figure 67.2 MR: A three-month-old boy with combined immunodeficiency. He had failed to thrive, had sparse facial and scalp hair, and had begun to have repeated infections. (Courtesy of Dr R Keightly of the University of Alabama.)



Figure 67.3 MR: At ten months of age, nine months following transplantation with fetal liver. He was thriving and looked well. (Courtesy of Dr R Keightly of the University of Alabama.)

method employs polymerase chain reaction to detect T-cell receptor excision circles (TRECs) that are pieces of DNA that occur during T-cell development.

TREATMENT

The current definitive treatment of ADA deficiency is bone marrow transplantation. The first survivors of SCID due to ADA deficiency were those that had been successfully treated with bone marrow transplantation [65] or with transplantation from fetal liver (Figures 67.2 and 67.3). Successful treatment by bone marrow transplantation has readily been accomplished when a histocompatible donor has been available; or, in the absence of such a donor, with half-matched parental marrow after removal of most of the post-thymic T cells [66]. In the presence of histocompatibility antigens (HLA)-identical bone marrow, the engraftment rate is around 80 percent, and full immune repopulation occurs in approximately six months [64]. In experience with patients with SCID, four of whom were ADA-deficient, all received HLA-identical bone marrow grafts and survived [67].

It has been found that transfusion of frozen irradiated red blood cells from normal individuals provided circulating levels of ADA and restored normal and cell-mediated immunity [27, 68, 69]. Levels of dATP were reduced [69]. The half-life of transfused ADA activity is 30 days and so treatment must be repeated every 4 weeks.

These observations led to the development of enzyme replacement therapy using bovine ADA conjugated to polyethyleneglycol (PEG-ADA), which has proved to be useful for many patients [24, 70]. It is given initially twice a week and later weekly as an intramuscular injection. PEG-ADA treatment restores immune competence. PEG-ADA has been employed to prepare very ill patients for transplantation.

ADA deficiency has also been treated by gene therapy [71, 72]. Cells isolated from autologous peripheral blood of the patient's cord blood have been used as recipients for transfer of a viral vector containing the human ADA gene and then infused into the patient. In others, treatment was with repeated infusions of transduced peripheral mononuclear cells or stem cells from the marrow. Patients have now been reported [73, 74] with successful reconstitution of immunity in ADA deficiency in the absence of PEG-ADA with follow up of two and four years.

REFERENCES

- Giblett ER, Anderson JE, Cohen F *et al.* Adenosine deaminase deficiency in two patients with severely impaired cellular immunity. *Lancet* 1972; **2**: 1067.
- Giblett ER. ADA and PNP deficiencies: how it all began. *Ann NY Acad Sci* 1985; **451**: 1.
- Pollara B, Pickering RJ, Meuwissen HJ. Combined immunodeficiency disease associated with adenosine deaminase deficiency an inborn error of metabolism. *Pediatr Res* 1973; **7**: 362.
- Giblett ER, Ammann AJ, Wara DW *et al.* Nucleoside-phosphorylase deficiency in a child with severely defective T-cell immunity and normal B-cell immunity. *Lancet* 1975; **1**: 1010.
- Hirschhorn R. Overview of biochemical abnormalities and molecular genetics of adenosine deaminase deficiency. *Pediatr Res* 1993; **33**(Suppl. 1): S35.
- Petersen MB, Tranebjærg L, Tommerup N *et al.* New assignment of the adenosine deaminase gene locus to chromosome 20q1311 by study of a patient with interstitial deletion 20q. *J Med Genet* 1987; **24**: 93.
- Wiginton DA, Kaplan DJ, States JC *et al.* Complete sequence and structure of the gene for human adenosine deaminase. *Biochemistry* 1986; **25**: 8234.
- Hirschhorn R, Ellenbogen A, Tzall S. Five missense mutations at the adenosine deaminase locus (ADA) detected by altered restriction fragments and their frequency in ADA: patients with severe combined immunodeficiency (ADA-SCID). *Am J Med Genet* 1992; **42**: 201.
- Markert ML. Molecular basis of adenosine deaminase deficiency. *Immunodeficiency* 1994; **5**: 141.
- Arredondo-Vega FX, Santisteban I, Richard B *et al.* Adenosine deaminase deficiency with mosaicism for a 'second-site suppressor' of a splicing mutation: decline in revertant T lymphocytes during enzyme replacement therapy. *Blood* 2002; **99**: 1005.
- Meuwissen HJ, Pollara B, Pickering RJ. Combined immunodeficiency disease associated with adenosine deaminase deficiency. *J Pediatr* 1975; **86**: 169.
- Dissing J, Knudsen B. Adenosine-deaminase deficiency and combined immunodeficiency syndrome. *Lancet* 1972; **2**: 1316.
- Ochs HD, Yount JE, Giblett ER *et al.* Adenosine-deaminase deficiency and severe combined immunodeficiency syndrome. *Lancet* 1973; **1**: 1393.
- Parkman R, Gelfand EW, Rosen FS *et al.* Severe combined immunodeficiency and adenosine deaminase deficiency. *N Engl J Med* 1975; **292**: 714.
- Morgan G, Levinsky RJ, Hugh-Jones K *et al.* Heterogeneity of biochemical clinical and immunological parameters in severe combined immunodeficiency due to ADA deficiency. *Clin Exp Immunol* 1987; **70**: 491.
- Shovlin CL, Simmonds HA, Fairbanks LD *et al.* Adult onset immunodeficiency caused by inherited adenosine deaminase deficiency. *J Immunol* 1994; **153**: 2331.
- Borkowsky W, Gershon AA, Shenkman LS, Hirschhorn R. Adenosine deaminase deficiency without immunodeficiency: clinical and metabolic studies. *Pediatr Res* 1980; **14**: 885.
- van Gennip AH, van Cruchten AG, Bootsma AH *et al.* Detection of patients with adenosine deaminase deficiency by HPLC/ESI tandem-MS analysis of blood spots. *J Inher Metab Dis* 2002; **25**(Suppl. 1): 156.
- Hirschhorn R. Incidence and prenatal detection of adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency. In: Pollara B, Pickering RJ, Meuwissen HJ, Porter IH (eds). *Inborn Errors of Specific Immunity*. New York: Academic Press, 1979: 5.
- Stephan JL, Vlekova V, Le Deist F *et al.* A retrospective single center study of clinical presentation and outcome in 117 patients. *J Pediatr* 1993; **123**: 564.
- Shovlin CL, Hughes JMB, Simmonds HA *et al.* Adult presentation of adenosine deaminase deficiency. *Lancet* 1993; **341**: 1471.
- Hong R, Gatti R, Rathbun JC, Good RA. Thymic hypoplasia and thyroid dysfunction. *N Engl J Med* 1970; **282**: 470.
- Geffner ME, Stiehm ER, Stephure D, Cowan MJ. Probable autoimmune thyroid disease and combined immunodeficiency disease. *Am J Dis Child* 1986; **140**: 1194.
- Hershfield MS, Chaffee S, Sorensen RU. Enzyme replacement therapy with polyethylene glycol-adenosine deaminase in adenosine deaminase deficiency: overview and case reports of three patients including two now receiving gene therapy. *Pediatr Res* 1993; **33**(Suppl.): 42.
- Hirschhorn R. Adenosine deaminase deficiency. In: Rosen FS, Seligmann M (eds). *Immunodeficiency Reviews*. New York: Harwood Academic, 1990: 175.
- Hirschhorn R, Papageorgiou PS, Kesariwala HH, Taft LT. Amelioration of neurologic abnormalities after 'enzyme replacement' in adenosine deaminase deficiency. *N Engl J Med* 1980; **303**: 377.
- Polmar SH, Stern RC, Schwartz AL *et al.* Enzyme replacement therapy for adenosine deaminase deficiency and severe combined immunodeficiency. *N Engl J Med* 1976; **295**: 1337.
- Tanaka C, Hara T, Suzaki I *et al.* Sensorineural deafness in siblings with adenosine deaminase deficiency. *Brain Dev* 1996; **18**: 304.
- Rogers MH, Lwin R, Fairbanks L *et al.* Cognitive and behavioural abnormalities in adenosine deaminase deficient severe combined immunodeficiency. *J Pediatr* 2001; **139**: 44.
- Albuquerque W, Gaspar H. Bilateral sensorineural deafness in adenosine deaminase-deficient severe combined immunodeficiency. *J Pediatr* 2004; **144**: 278.

31. Ozsahin H, Arredondo-Vega FX, Santisteban I *et al.* Adenosine deaminase (ADA) deficiency in adults. *Blood* 1997; **89**: 2849.
32. Buckley RH, Schiff RI, Schiff SE *et al.* Human severe combined immunodeficiency: genetic phenotypic and functional diversity in one hundred eight infants. *J Pediatr* 1997; **130**: 378.
33. Wolfson JJ, Cross VF. The radiographic findings in 49 patients with combined immunodeficiency. In: HJ Meuwissen HJ, Pickering RJ, Pollara B, Porter IH (eds). *Combined Immunodeficiency Disease and Adenosine Deaminase Deficiency. A Molecular Defect*. New York: Academic Press, 1975: 225.
34. Shovlin CL, Simmonds HA, Fairbanks LD *et al.* Adult onset immunodeficiency caused by inherited adenosine deaminase deficiency. *J Immunol* 1994; **153**: 2331.
35. Husain M, Grunebaum E, Naqvi A *et al.* Burkitt's lymphoma in a patient with adenosine deaminase deficiency-severe combined immunodeficiency treated with polyethyleneglycol adenosine deaminase. *J Pediatr* 2007; **151**: 93.
36. Huber J, Kersey J. Pathological findings. In: HJ Meuwissen HJ, Pickering RJ, Pollara B, Porter IH (eds). *Combined Immunodeficiency Disease and Adenosine Deaminase Deficiency. A Molecular Defect*. New York: Academic Press, 1975: 279.
37. Hirschhorn R, Beratis N, Rosen FS *et al.* Adenosine deaminase deficiency in a child diagnosed prenatally. *Lancet* 1975; **1**: 73.
38. Agarwal RP, Crabtree GW, Parks RE Jr *et al.* Purine nucleoside metabolism in the erythrocytes of patients with adenosine deaminase deficiency and severe combined immunodeficiency. *J Clin Invest* 1976; **57**: 1025.
39. Scott CR, Chen SH, Giblett ER. Detection of the carrier state in combined immunodeficiency disease associated with adenosine deaminase deficiency. *J Clin Invest* 1974; **53**: 1194.
40. Moore EC, Meuwissen HJ. Screening for ADA deficiency. *J Pediatr* 1974; **95**: 802.
41. Hershfield MS. Genotype is an important determinant of phenotype in adenosine deaminase deficiency. *Curr Opin Immunol* 2003; **15**: 571.
42. Dooley T, Fairbanks LD, Simmonds HA *et al.* First trimester diagnosis of adenosine deaminase deficiency. *Prenat Diagn* 1987; **7**: 561.
43. Ziegler JB, Van der Weyden MB, Lee CH, Daniel A. Prenatal diagnosis for adenosine deaminase deficiency. *J Med Genet* 1981; **18**: 154.
44. Schrader WP, Stacy AR. Purification and subunit structure of adenosine deaminase from human kidney. *J Biol Chem* 1977; **252**: 6409.
45. Gossage DL, Norby-Slycord CJ, Hershfield MS, Markert ML. A homozygous 5 base-pair deletion in exon 10 of the adenosine deaminase (ADA) gene in a child with severe combined immunodeficiency and very low levels of ADA mRNA and protein. *Hum Mol Genet* 1993; **2**: 1493.
46. Santisteban I, Arredondo-Vega FX, Kelly S. Novel splicing missense and deletion mutations in seven adenosine deaminase-deficient patients with late/delayed onset of combined immunodeficiency disease. Contribution of genotype to phenotype. *J Clin Invest* 1993; **92**: 2291.
47. Yang DR, Huie ML, Hirschhorn R. Homozygosity for a missense mutation (G20R) associated with neonatal onset adenosine deaminase-deficient severe combined immunodeficiency (ADA-SCID). *Clin Immunol Immunopathol* 1994; **70**: 171.
48. Hirschhorn R, Yang DR, Insel RA, Ballow M. Severe combined immunodeficiency of reduced severity due to homozygosity for an adenosine deaminase missense mutation (Arg253Pro). *Cell Immunol* 1993; **152**: 383.
49. Cooper DN, Krawczak D. The mutational spectrum of single base-pair substitutions causing human genetic disease: patterns and predictions. *Hum Genet* 1990; **85**: 55.
50. Cooper DN, Youssoufian H. The CpG dinucleotide and human genetic disease. *Hum Genet* 1988; **78**: 151.
51. Hirschhorn R, Yang DR, Israni A *et al.* Somatic mosaicism for a newly identified splice-site mutation in a patient with adenosine deaminase-deficient immunodeficiency and spontaneous clinical recovery. *Am J Hum Genet* 1994; **55**: 59.
52. Arredondo-Vega FX, Santisteban I, Kelly S *et al.* Correct splicing despite a G A mutation at the invariant first nucleotide of a 59 splice site: a possible basis for disparate clinical phenotypes in siblings with adenosine deaminase (ADA) deficiency. *Am J Hum Genet* 1994; **54**: 820.
53. Brinkmann B, Brinkmann M, Martin H. A new allele in red cell adenosine deaminase polymorphism: ADA0. *Hum Hered* 1973; **23**: 603.
54. De La Haba G, Cantoni GL. The enzymatic synthesis of S-adenosyl-L-homocysteine from adenosine and homocysteine. *J Biol Chem* 1959; **234**: 603.
55. Naylor EW, Orfanos AP, Guthrie R. An improved screening test for adenosine deaminase deficiency. *J Pediatr* 1978; **93**: 473.
56. Coleman MS, Donofrio J, Hutton JJ *et al.* Identification and quantitation of adenine deoxynucleotides in erythrocytes of a patient with adenosine deaminase deficiency and severe combined immunodeficiency. *J Biol Chem* 1978; **253**: 1619.
57. Cohen A, Hirschhorn R, Horowitz SD *et al.* Deoxyadenosine triphosphate as a potentially toxic metabolite in adenosine deaminase deficiency. *Proc Natl Acad Sci USA* 1978; **75**: 472.
58. Henderson JF, Scott FW, Lowe JK. Toxicity of naturally occurring purine deoxyribonucleotides. *Pharmacol Ther* 1980; **8**: 573.
59. Matsumoto SS, Yu AL, Bleeker LC *et al.* Biochemical correlates of the differential sensitivity of subtypes of human leukemia to deoxyadenosine and deoxycoformycin. *Blood* 1982; **60**: 1096.
60. Carson DA, Kaye J, Seegmiller JE. Differential sensitivity of human leukemic T-cell lines and B-cell lines to growth inhibition by deoxyadenosine. *J Immunol* 1978; **121**: 1726.
61. Seto S, Carrera CJ, Kubota M *et al.* Mechanism of deoxyadenosine and 2-chlorodeoxyadenosine toxicity to nondividing human lymphocytes. *J Clin Invest* 1985; **75**: 377.
62. Hershfield MS. Apparent suicide inactivation of human lymphoblast S-adenosylhomocysteine hydrolase by 29-deoxyadenosine and adenine arabinoside. A basis for direct toxic effects of analogs of adenosine. *J Biol Chem* 1979; **254**: 22.
63. Stiles GL. Adenosine receptors: structure function and regulation. *Trends Physiol Sci* 1986; **7**: 486.

-
64. Kuehn B. State, federal efforts underway to identify children with 'Bubble Boy Syndrome'. *J Am Med Assoc* 2010; **304**: 16.
65. Meuwissen HJ, Moore E, Pollara B. Maternal marrow transplant in a patient with combined immunodeficiency disease (CID) and adenosine deaminase (ADA) deficiency. *Pediatr Res* 1973; **7**: 362.
66. Buckley RH. Breakthroughs in the understanding and therapy of primary immunodeficiency. *Pediatr Clin North Am* 1994; **41**: 665.
67. Buckley RH, Schiff SE, Schiff RI *et al*. Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. *N Engl J Med* 1999; **340**: 508.
68. Dyminski JW, Daoud A, Lampkin BC *et al*. Immunological and biochemical profiles in response to transfusion therapy in an adenosine-deaminase deficient patient with severe combined immunodeficiency. *Clin Immunol Immunopathol* 1979; **14**: 307.
69. Donofrio J, Colmena MS, Hutton JJ *et al*. Overproduction of adenine deoxynucleosides and deoxynucleotides in adenosine deaminase deficiency with severe combined immunodeficiency disease. *J Clin Invest* 1978; **62**: 884.
70. Hershfield MS, Buckley RH, Greenberg ML *et al*. Treatment of adenosine deaminase deficiency with polyethylene glycol-modified adenosine deaminase. *N Engl J Med* 1987; **316**: 589.
71. Parkman R, Gelfand EW. Severe combined immunodeficiency disease adenosine deaminase deficiency and gene therapy. *Curr Opin Immunol* 1991; **3**: 547.
72. Blaese RM. Development of gene therapy for immunodeficiency: adenosine deaminase deficiency. *Pediatr Res* 1993; **33**(Suppl. 1): S49.
73. Gaspar HB, Bjorkegren E, Parsley K. Successful reconstitution of immunity in ADA-SCID by stem cell gene therapy following cessation of PEG-ADA and use of mild preconditioning. *Mol Ther* 2006; **4**: 506.
74. Aiuti A, Cattaneo F, Galimberti S *et al*. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. *N Engl J Med* 2009; **5**: 518.

Adenylosuccinate lyase deficiency

Introduction	514	Treatment	516
Clinical abnormalities	515	References	516
Genetics and pathogenesis	516		

MAJOR PHENOTYPIC EXPRESSION

Developmental impairment, seizures, autistic behavior, excretion of adenylosuccinate and succinylaminoimidazole-carboxamide riboside in the urine and deficient activity of adenylosuccinate lyase.

INTRODUCTION

Adenylosuccinate lyase (ASL) deficiency was first described by Jaeken and Van den Berghe [1] in 1984. This created enormous interest because autistic behavior was observed in the affected patients in this family, and it would be of considerable interest if it were possible to relate molecular changes in the gene for ASL to the genetics of autism [2]. However, extensive survey of autistic populations has failed to turn up additional patients with lyase deficiency. A more typical phenotype includes seizures and developmental delay [3].

The enzyme adenylosuccinate lyase (adenylosuccinase, ASL; EC 4.3.2.2) catalyzes the eighth step in the *de novo* synthesis of purines in which succinylaminoimidazolecarboxamide ribotide (SAICAR, SAICAMP) is converted to aminoimidazolecarboxamide ribotide (AICAR, AICAMP, ZMP) (Figure 68.1) [4, 5]. The same enzyme catalyzes the conversion of adenylosuccinate to adenosine monophosphate (AMP) in the cycle of purine nucleotide conversions that yield adenine nucleotides [6]. Deficient activity of the enzyme was documented in 1988 by Jaeken and colleagues [4]. The human gene has been mapped to chromosome 22q1.3.1.-1.3.2 [7]. The

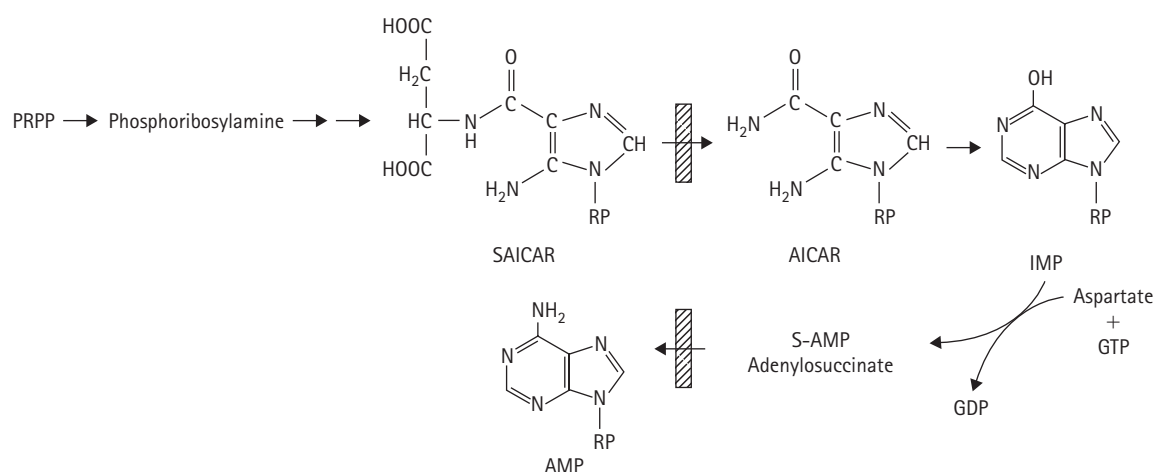


Figure 68.1 The reactions catalyzed by adenylosuccinate lyase. In the *de novo* pathway of purine nucleotide synthesis the conversion of 5-phosphoribosyl-5-amino-4-imidazole succinyl-carboxamide (SAICAR) to 5-phosphoribosyl-5-amino-4-imidazole carboxamide (AICAR) and in the purine interrelations cycle the conversion of adenylosuccinate to adenylic acid (AMP).

human cDNA has been cloned, and the nature of the point mutation was defined in the initial family reported [1, 2]. A majority of the 30 different mutations initially delineated were missense, most of them in compound heterozygotes. The most common mutation, p.R426H, was found in 17 families from many countries [3, 8]. More than 50 patients have now been reported [3]. Mutations have continued to be missense, many on single alleles.

CLINICAL ABNORMALITIES

The phenotype of adenylosuccinate lyase deficiency is variable, but it is clear that psychomotor impairment is a regular manifestation of the disease [1, 4, 6, 9, 10]. Many have had seizure disorders. Some of the patients with early onset seizures have died in infancy [11–15]. More have had moderate to severely impaired mental development and seizures after the first year [1, 4, 9, 16]. One patient experienced a fatal neonatal disease [3]. The infant died of respiratory failure having shown no spontaneous movements. Autistic features in some have included absence of eye contact, repetitive behavior, temper tantrums, and self-injurious behavior, none of them rare in individuals with impaired mental development. Impaired growth and muscle wasting have also been observed [4]. Three patients had only mild developmental delay [3, 4]. They have been referred to as type II to distinguish them from all the other patients in type I, in whom impaired mental development is more severe [17]. Another patient was described [9] as having an intermediate degree of symptomatology and another had only delayed motor development and severe hypotonia [18]. Siblings shown in Figure 68.2 had less severely impaired mental development [11]. It seems

likely that once a large number of patients is observed, a spectrum will be the case rather than discrete groups of phenotypes.

Facial dysmorphic features were reported [19] in two patients who had brachycephaly, a short nose with anteverted nares, a smooth philtrum, thin upper lip, brachycephaly, and low set ears.

A behavioral phenotype has been reported [20] resembling Angelman syndrome. Two sisters, 11 and 12 years of age, had global developmental delay, motor apraxia, and severe deficits in speech. They also had seizures. The behavior was characterized by happy dispositions and excessive laughter. They were hyperactive and had short attention spans. They mouthed objects and had tantrums and stereotypical movements. Self-injurious behavior has been observed in this disease [1, 3, 21].

A distinctive feature of ASL deficiency that simplifies the detection of this disorder is the accumulation of the metabolites adenylosuccinate (succinyladenosine) and SAICArriboside (succinyl-AICArriboside), the dephosphorylated products of the substrates for the deficient enzyme. It is possible to screen for the latter compound, because it gives a positive Bratton-Marshall reaction (Figure 68.3) [22]. Confirmation of a positive screening test is done by identification of adenylosuccinate and SAICArriboside in urine or blood [1]. Both compounds are also readily found in the cerebrospinal fluid, where concentrations are 20- to 100-fold those of plasma and are as high as 500 mmol/L [1, 23]. Urinary excretions range from 25 to 700 mmol/mol creatinine [1, 4, 23]. In most patients with the classic neonatal phenotype, the ratio of the two compounds adenylosuccinate/succinyl-AICArriboside in the cerebrospinal fluid (CSF) approximates 1 [3, 10]. Patients with milder phenotypes have had more adenylosuccinate,



Figure 68.2 Two siblings with adenylosuccinate lyase deficiency, shown with their parents. Their degree of mental impairment was described as less severe. (This illustration was kindly provided by Dr Ivan Sebesta of Universita Karlova, Prague, Czech Republic.)

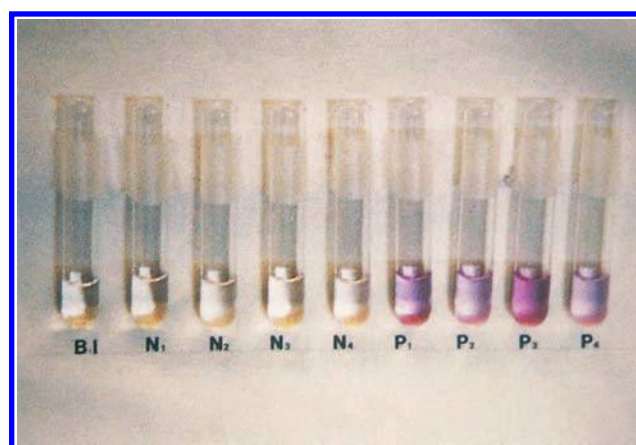


Figure 68.3 The Bratton-Marshall reaction. On the left are negative tubes; on the right, the urine of two patients with adenylosuccinate lyase deficiency.

sometimes as much as four-fold higher or even 100-fold [4], and the ratio is over 2. In the severe neonatal disease, the ratio is less than 1.

GENETICS AND PATHOGENESIS

The disorder is inherited as an autosomal recessive trait. Consanguinity has been described [1]. The enzyme has been generally assayed by following the conversion of adenylosuccinate to AMP spectrophotometrically [24]. Liver, fibroblasts, and lymphocytes have been used to document the deficiency of enzyme activity in individuals with ASL deficiency [4, 10, 25, 26]. The very different metabolite ratio in the type II patient suggested that the enzyme be assayed with both substrates. It was found that in classic type I ASL deficiency, the activities of the enzyme toward the two substrates are decreased in parallel to about 30 percent of control [9]. In general, the activity of mutant enzymes against both substrates are proportionately decreased, regardless of clinical phenotype [27]. In one study of fibroblasts derived from a patient with a type II genotype, activity against succinylAICAR was about 30 percent of control, but when adenylosuccinate was the substrate, the activity was only 3 percent of control [10], data consistent with the higher concentration of adenylosuccinate in the type II patient.

Kinetic studies have indicated that in lymphoblasts [28], as well as in fibroblasts [10], the affinity for adenylosuccinate is normal even at physiological temperatures [2]. Furthermore, the variant enzyme has been shown to have decreased stability to heat [28], and this would be consistent with the reduced residual activity. An increase in the K_m for succinylAICAR in some patients [10] indicates modification of the active site. The kinetics of the type II enzyme differed in that the K_m for adenylosuccinate was markedly increased [10]; its V_{max} was strongly inhibited by KCl and nucleoside triphosphates, neither of which affected the kinetics of type I. Mammalian ASL is a heteropolymer of about 52 kDa containing four subunits [29].

The cDNA for the gene contains 1452 nucleotides and codes for a protein of 484 amino acids [27]. Molecular analysis in the first reported Moroccan family with four affected children [1, 2, 4] indicated homozygosity for a point mutation in the ASL gene resulting in a serine to proline change originally placed at amino acid 413, but now called S438P in the 484 amino acid protein. This might be expected to increase the flexibility of the peptide backbone of the enzyme, which might account for decreased stability. Analysis of genomic polymerase chain reaction (PCR) products from the parents of the patients revealed both a normal and a mutant allele, documenting heterozygosity [2]. The mutation p.R426H remains the most common mutation in a variety of unrelated patients [3]. Another, p.Y114H, has also been found in more than one unrelated family [3]. Most mutations continue to be private. Another

mutation identified [30] in a gypsy patient without known consanguinity, G1279A, converted a well-conserved arginine at 401 to histidine. Other missense mutations identified have indicated a high degree of molecular heterogeneity [30–32]. A 39-bp deletion in the cDNA was caused by a C to A change in exon 5 creating a consensus 59 donor splice site [33]. One nonsense mutation has been observed [27].

An interesting mutation was found in three unrelated patients [8]. The coding sequence was normal in the allele with the mutation, which was a c.49T>C change in the 5'-untranslated region (UTR). This led to a reduction to about 25 percent of wild-type promoter function and mRNA. The mutation affected the binding of a known activator of transcription, nuclear regulatory factor 2 (NRF-2). These observations are consistent with a role for NRF-2 in the regulation of purine synthesis.

Despite the continuing discovery of novel mutations [34], correlations between genotype and phenotype have been elusive.

TREATMENT

Specific therapy has not been devised. Seizures may be treated with the usual anticonvulsant drugs. Management is designed for optimal developmental potential. A 12-month trial of ribose therapy was without effect [3].

REFERENCES

1. Jaeken J, Van den Berghe G. An infantile autistic syndrome characterized by the presence of succinylpurines in body fluids. *Lancet* 1984; **2**: 1058.
2. Stone RL, Aimi J, Barshop BA *et al*. A mutation in adenylosuccinate lyase associated with mental retardation and autistic features. *Nat Genet* 1992; **1**: 59.
3. Jurecka A, Zikanova M, Tylki-Szymska A *et al*. Clinical, biochemical and molecular findings in seven Polish patients with adenylosuccinate layase deficiency. *Mol Genet Met* 2008; **94**: 435.
4. Jaeken J, Wadman SK, Duran M *et al*. Adenylosuccinase deficiency: an inborn error of purine nucleotide synthesis. *Eur J Pediatr* 1988; **148**: 126.
5. Lowy BA, Ben-Zion D. Adenylosuccinase activity in human and rabbit erythrocyte lysates. *J Biol Chem* 1970; **245**: 3043.
6. Van den Berghe G, Bontemps F, Vincent MF, Van den Bergh F. The purine nucleotide cycle and its molecular defects. *Prog Neurobiol* 1992; **39**: 547.
7. Fon EA, Demczuk S, Delattre O *et al*. Mapping of the human adenylosuccinate lyase (ADSL) gene to chromosome 22q131-q132. *Cytogenet Cell Genet* 1993; **64**: 201.
8. Marie S, Race V, Nassogne M-C *et al*. Mutation of a nuclear respiratory factor 2 binding site in the 5' untranslated region of the ADSL gene in three patients with adenylosuccinate lyase deficiency. *Am J Hum Genet* 2002; **71**: 14.

9. Jaeken JF, Van der Bergh F, Vincent MF *et al.* Adenylosuccinase deficiency: a newly recognized variant. *J Inherit Metab Dis* 1992; **15**: 416.
10. Van den Bergh F, Vincent MF, Jaeken J, Van den Berghe G. Residual adenylosuccinase activities in fibroblasts of adenylosuccinase deficient children: parallel deficiency with adenylosuccinate and succinyl-AICAR in profoundly retarded patients and on-parallel deficiency in a mildly retarded girl. *J Inherit Metab Dis* 1993; **16**: 415.
11. Sebesta I, Krijt J, Kmoch S *et al.* Adenylosuccinase deficiency – clinical and biochemical findings in 5 Czech patients. *J Inherit Metab Dis* 1996; **19**: 2 (Abstr. 04).
12. Van den Bergh FAJTM, Boschaart AN, Hageman G *et al.* Adenylosuccinase deficiency with neonatal onset severe epileptic seizures and sudden death. *Neuropediatrics* 1998; **29**: 51.
13. Krijt J, Sebesta I, Svehlakova A *et al.* Adenylosuccinate lyase deficiency in a Czech girl and two siblings. *Adv Exp Med Biol* 1995; **370**: 367.
14. Maaswinkel-Mooij PD, Laan LAEM, Onkenhout W *et al.* Adenylosuccinase deficiency presenting with epilepsy in early infancy. *J Inherit Metab Dis* 1997; **20**: 606.
15. Köhler M, Assmann B, Bräutigam C *et al.* Adenylosuccinase deficiency: possibly underdiagnosed encephalopathy with variable clinical features. *Eur J Pediatr Neurol* 1999; **3**: 6.
16. Salerno C, Crifo C, Giardini O. Adenylosuccinase deficiency: a patient with impaired erythrocyte activity and anomalous response to intravenous fructose. *J Inherit Metab Dis* 1995; **18**: 602.
17. Van den Berghe G, Vincent MF, Jaeken J. Inborn errors of the purine nucleotide cycle: adenylosuccinase deficiency. *J Inherit Metab Dis* 1997; **20**: 193.
18. Valik D, Miner PT, Jones JD. First US case of adenylosuccinate lyase deficiency with severe hypotonia. *Pediatr Neurol* 1997; **16**: 252.
19. Holder-Espinasse M, Bourrouillou G, Ceballos-Picot I *et al.* Towards a suggestive facial dysmorphism in adenylosuccinate lyase deficiency? *J Med Genet* 2002; **39**: 440.
20. Gitiaux C, Ceballos-Picot I, Valayannopoulos V *et al.* Misleading behavioural phenotype with adenylosuccinate lyase deficiency. *Eur J Hum Genet* 2009; **17**: 133.
21. Sebesta I, Krijt J, Kmoch S *et al.* Adenylosuccinase deficiency: clinical and biochemical findings in 5 Czech patients. *J Inherit Metab Dis* 1997; **20**: 343.
22. Laikind PK, Seegmiller JE, Gruber HE. Detection of 59-phosphoribosyl-4-(N-succinylcarboxamide)-5-aminoimidazole in urine by use of the Bratton–Marshall reaction: identification of patients deficient in adenylosuccinate lyase activity. *Anal Biochem* 1986; **156**: 81.
23. De Bree PK, Wadman SK, Duran M, Faabery de Jonge H. Diagnosis of inherited adenylosuccinase deficiency by thin-layer chromatography of urinary imidazoles and by automated cation exchange column chromatography of purines. *Clin Chim Acta* 1986; **156**: 279.
24. Schultz V, Lowenstein JM. Purine nucleotide cycle. Evidence for the occurrence of the cycle in brain. *J Biol Chem* 1976; **251**: 485.
25. Van der Bergh F, Vincent MF, Jaeken J, Van den Berghe G. Radiochemical assay of adenylosuccinase: demonstration of parallel loss of activity toward both adenylosuccinate and succinylaminoimidazole carboxamide ribotide in liver of patients with the enzyme defect. *Anal Biochem* 1991; **193**: 287.
26. Van den Berghe G, Jaeken J. Adenylosuccinase deficiency. *Adv Exp Med Biol* 1986; **195A**: 27.
27. Kmoch S, Hartmannová H, Stiburková B *et al.* Human adenylosuccinate lyase (ADSL) cloning and characterization of full-length cDNA and its isoform gene structure and molecular basis for ADSL deficiency in six patients. *Hum Mol Genet* 2000; **9**: 1501.
28. Barshop BA, Alberts AS, Gruber HE. Kinetic studies of mutant human adenylosuccinase. *Biochim Biophys Acta* 1989; **999**: 19.
29. Casey PJ, Lowenstein JM. Purification of adenylosuccinate lyase from rat skeletal muscle by a novel affinity column. Stabilization of the enzyme and effects of anions and fluoro analogues of the substrate. *Biochem J* 1987; **246**: 263.
30. Kmoch S, Hartmannová H, Krijt J, Sebesta I. Adenylosuccinase deficiency – identification of a new disease causing mutation. *J Inherit Metab Dis* 1996; **19**: 13.
31. Kmoch S, Hartmannová H, Krijt J *et al.* Genetic heterogeneity in adenylosuccinate lyase deficiency. *Clin Biochem* 1997; **30**: 22.
32. Verginelli D, Luckow B, Crifo C *et al.* Identification of new mutations in the adenylosuccinate lyase gene associated with impaired enzyme activity in lymphocytes and red blood cells. *Biochim Biophys Acta* 1998; **1406**: 81.
33. Marie S, Cuppens H, Heutenspreute M *et al.* Mutation analysis in adenylosuccinate lyase deficiency. Eight novel mutations in the re-evaluated full ADSL coding sequence. *Hum Mutat* 1999; **13**: 197.
34. Spiegel EK, Colman RF, Patterson D. Adenylosuccinate lyase deficiency. *Mol Genet Metab* 2006; **89**: 19.

Orotic aciduria

Introduction	518	Treatment	520
Clinical features	518	References	520
Genetics and pathogenesis	519		

MAJOR PHENOTYPIC EXPRESSION

Megaloblastic anemia, failure to thrive, susceptibility to infection, crystalluria, orotic aciduria, and deficient activity of orotidylic pyrophosphorylase and orotidylic decarboxylase.

INTRODUCTION

Orotic aciduria was first reported by Huguley *et al.* in 1959 [1] in a single patient who illustrated clearly the features of the disease. The rarity of the disease is indicated by the fact that it was 1965 before a second patient was described [2]. The importance of these two papers is reflected not only in the thoroughness of the clinical and metabolic documentation, but by the fact that they set out the definitive treatment with uridine [2,3] which has been extraordinarily effective and serves as a model for the fact that the thorough understanding of the nature of a fundamental defect can lead to rational and effective treatment.

The disease is also special as an example of a defect in a single autosomal recessive gene causing defective activity of two sequential enzymes in the *de novo* pathway of pyrimidine nucleotide biosynthesis (Figure 69.1), orotidylic (OMP) pyrophosphorylase (OPRT) (EC 2.4.2.10) and

orotidylic (OMP) decarboxylase (EC 4.1.1.23) [4, 5]. The gene on chromosome 3 has been sequenced, and a small number of mutations have been defined [6].

CLINICAL FEATURES

The classic presentation (Table 69.1) [1, 2, 7] is with failure to thrive, but these infants are found to be pale and anemic on initial examination [1, 2, 5]. A few young infants presented with anemia in which growth had to date (two months) been normal [8]. A seven-year-old who developed symptoms of anemia at six years of age, but who had apparently been pale previously, had normal growth and intellectual development [9]. In the classic presentation, hair is sparse, fine, and very short [2]. Nails have grown poorly. One patient treated at 19 months with uridine had not had his nails trimmed for the previous six months [2].

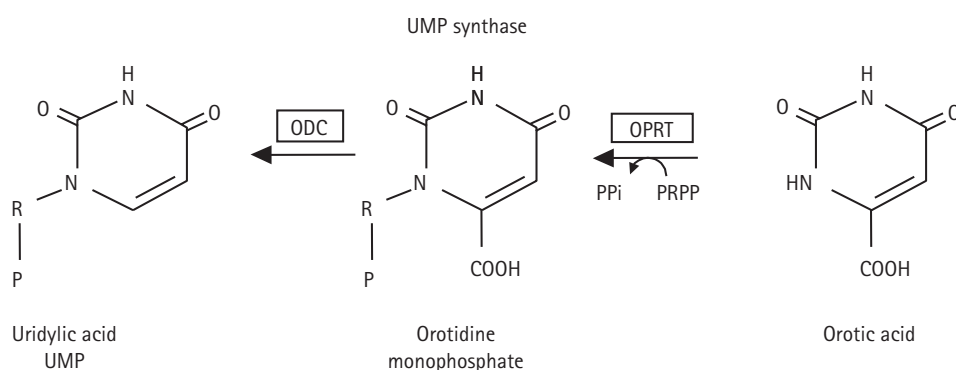


Figure 69.1 The pathway of pyrimidine nucleotide synthesis illustrating the enzymes OPRT and OMP decarboxylase components of UMP synthase that are defective in orotic aciduria.

Table 69.1 Orotic aciduria clinical manifestations

Megaloblastic anemia	Crystalluria
Leukopenia	Hematuria
Susceptibility to infection	Obstructive uropathy
Failure to thrive	Urinary tract infection
Diarrhea	Nephropathy
Cognitive and motor developmental delay	

The anemia is characteristically megaloblastic [1, 3, 5, 7–10]. Neutropenia is present in most patients. Hemoglobin levels have often been 7–8 g/dL, hematocrit approximating 25 percent, but some have had more severe anemia [10]. Some had required transfusions. Red cell morphology has been unusual, with a marked degree of anisocytosis and poikilocytosis [2], macrocytosis and many strikingly large and oval shapes with long diameters. Many macrocytes were hypochromic, while levels of iron are normal, or increased [5]. Occasional polychromatic cells have been seen, as well as strippled cells, Howell–Jolly bodies, Cabot rings, and nucleated erythrocytes. Multisegmented neutrophils and giant platelets have been observed [2]. Bone marrow aspirates reveal megaloblastic changes in a majority of the nucleated red cells. The myelo/erythroid ratio may be reversed to 1:2 or 1:4. Giant myelocytes and metamyelocytes are also seen.

Concentrations of B₁₂ and folic acid are normal, and early patients were treated with B₁₂ and folate without effect [1, 2]. Gastric aspirates did show achlorhydria, some of them responsive to histamine [2, 8] and some not [2].

The urine appears normal as passed, but on standing, especially in the cold, a large, white precipitate forms [1, 2, 8, 11]. Crystals of orotic acid may be visible under the microscope [2]. The disease was identified as an inborn error of metabolism by the recruitment by Huguley *et al.* [1] of a biochemist, J Bain, who isolated and purified the crystals and determined that they were orotic acid.

The crystalluria may lead to gross or microscopic hematuria. One patient [9] came to attention because of hematuria and was found to be anemic. Proteinuria is unusual, but has been found, along with urinary tract infection [9]. Oliguria may accompany infection, or decreased fluid intake or dehydration from some other source such as diarrhea [1]. Under such circumstances, there may be urinary tract obstruction because of orotic acid sludging in the ureters or urethra [1]. An intravenous pyelogram revealed a nephrogram effect with radiopaque material remaining in the kidney 1.5 hours after injection, presumably because of obstruction of the renal collecting tubules by crystalline orotic acid [8]. Urethral obstruction has been successfully relieved by catheterization [4]. Urea nitrogen and creatinine in blood are usually normal, but will rise in the presence of obstruction.

Susceptibility to infection may be striking [1]. The initial patient had repeated respiratory infections and



Figure 69.2 HA: A 9-year-old with orotic aciduria and UMP synthase deficiency. She had no abnormal hematologic findings. She had mild developmental delay, predominately language, an abnormal EEG and episodes of cyclic vomiting and dehydration.

chronic diarrhea. He died of overwhelming varicella at 2.5 years [1]. An unusual feature was deficiency of specific immunoglobulins in one patient [8]. Others have had immunodeficiency, diarrhea, and stomatitis [12].

Congenital anomalies may or may not be a feature of this disease. Three of the first four patients reported had strabismus [2, 4, 7, 8] and one of these [7] had congenital heart disease. Another [4] had abnormal thoracic and abdominal musculature, herniation of lung into the supraclavicular region, umbilical and bilateral inguinal hernias, kyphoscoliosis, and a scaphoid skull. He also had hypertonia. Also, the published picture showed prominent genu recurvatum, and everted dorsiflexed feet in an exaggerated ballet first position.

Impaired physical and intellectual development has been observed [8, 13], but not invariably, since treatment has become available [3]. It has recently become apparent that there is another phenotype in which patients display developmental delay, in some relatively mild and no hematologic symptoms (Nyhan, unpublished observations) [12] (Figure 69.2). One had oculomotor apraxia. Orotic aciduria may be quantitatively less than in classic patients.

The orotic aciduria is usually massive. Excretion of as much as 1.34 and 1.5 g of orotic acid in 24 hours in a very young infant is not unusual [2]. The values approximate 1000 times the normal adult mean of 1.4 mg/24 hours. Orotidine excretion may also be elevated (15.8 mg/24 hours) as compared with the normal value of 2.5 mg/24 hours [8].

GENETICS AND PATHOGENESIS

The disease has been known to be autosomal recessive since early studies on four generations of heterozygous

relatives of the first patient [14] in whom enzyme activity was demonstratively reduced.

The enzymatic deficiency of OPRT and OMP decarboxylase can be demonstrated in erythrocytes, leukocytes, and cultured fibroblasts [4, 5, 7], as well as in liver [5]. Heterozygotes have intermediate level of activity, but they cannot always be reliably distinguished from control [4]. Initial distinction of types I and II orotic aciduria because of a patient in whom activity of OPRT appeared to be normal are clearly artificial in view of the fact that there is one gene and two enzymes. In fact, these early studies indicated that there were clear coordinate, straight-line relationships between the activities of the two enzymes in normal individuals and in patients [3]. Activities of enzymes earlier in the pathway, aspartate transcarbamylase and dihydroorotase, are elevated.

The defective enzyme, uridine-5-monophosphate (UMP) synthase contains in one polypeptide coded for by a single gene [6, 14] the activities of the two enzymes, which catalyze the last two steps of UMP synthesis [15].

The UMP synthase gene has been localized to chromosome 3q13 [16]. The gene contains six exons spanning approximately 15 kb. The protein contains 480 amino acids and has a molecular weight of 52,199. The two enzyme activities reside in distinct domains. The C-terminal 258 amino acids contain the decarboxylase and the N-terminal 214 the OPRT.

Two patients had a C378T missense mutation (P92S); in one a T961A mutation was also missense (I286N). In another family, two alleles contained R96G and G429A on one allele and V109G on the other [17]. Expression of human cDNAs containing these mutations in pyrimidine auxotrophic *Escherichia coli* demonstrated impaired enzyme activity. The patient with no megaloblastic anemia had a T928G mutation (I310V) [13].

TREATMENT

Orotic aciduria represents pyrimidine nucleotide starvation in man. It appears to be the first human nutritional auxotrophic disease to be recognized. The therapeutic effect of uridine is supportive of this hypothesis.

Excellent remission has regularly been obtained with doses of 50–300 mg/kg per day; some patients relapsed with less than 100 mg/kg, and only one required more than 200 mg/kg [12, 18, 19]. The dose most commonly employed is 150 mg/kg. Hematologic response is accompanied by weight gain and improvement in activity and well-being. Hair grows, and so do the nails. Orotic aciduria has not yet been treated with triacetyluridine, but it would doubtless be effective because its oral bioavailability is higher.

The conceptualization of this effective replacement therapy began with the first publication [1]. Administration of uridylic and cytidylic acids led to reduction in orotic acid excretion. This was presumably a consequence of

breakdown in the intestine to uridine and cytidine, as oral bioavailability of nucleotides is very low, and their administration usually results in diarrhea. Uridine therapy was initiated by Becroft and Phillips in the second patient [2]. Treatment begun at 16 months with 1.5 g/day led to a prompt rise in hemoglobin and a normal bone marrow. Activity and interest in his surroundings improved immediately, as did appetite. Hair and nails began to grow, as did he, crossing percentile lines for weight from below the 3rd percentile to between the 90th and 97th percentile. He remained mildly mentally impaired, but there was no progression. It was interesting that he experienced a prompt relapse on substitution of uracil for uridine, even though the content of pyrimidine base was twice that of uridine, which at that time was 75 mg/kg. Uridine therapy is dependent for bioavailability on efficient intestinal absorption and the activity of the salvage enzyme uridine kinase (EC2.7.1.48) which leads directly to the formation of the nucleotide UMP [20, 21].

6-Azaauridine and 6-azauracil, used in cancer chemotherapy, must be converted to their nucleotides for them to have antitumor activity; consistent with the lack of effect of uracil in orotic aciduria, 6-azauridine is 20 times more effective as an antitumor agent than azauracil [22]. Growth of fibroblasts of a patient with UMP synthase deficiency in medium containing 6-azauridine displayed nearly normal levels of the two defective enzymes [23]. These observations, that an enzyme inhibitor may be therapeutic depending on interaction with the structure of the mutant protein or protection from degradation, could lead to therapy with azauridine, but uridine therapy has been so effective, it has not been tried.

Activities of aspartate transcarbamylase and dihydroorotase, which are elevated in the untreated patient, decrease with uridine therapy.

REFERENCES

1. Huguley CM Jr, Bain JA, Rivers SL, Scoggins RB. Refractory megaloblastic anemia associated with excretion of orotic acid. *Blood* 1959; **14**: 615.
2. Becroft DM, Phillips LI. Hereditary orotic aciduria and megaloblastic anemia: a second case, with response to uridine. *Br Med J* 1965; **5434**: 547.
3. Becroft DM, Phillips LI, Simmonds A. Hereditary orotic aciduria: long-term therapy with uridine and a trial of uracil. *J Pediatr* 1969; **75**: 885.
4. Fox RM, O'Sullivan WJ, Firkin BG. Orotic aciduria. Differing enzyme patterns. *Am J Med* 1969; **47**: 332.
5. Smith LH, Sullivan M, Huguley CM. Pyrimidine metabolism in man. IV. The enzymatic defect of orotic aciduria. *J Clin Invest* 1961; **40**: 656.
6. Suttle DP, Bugg BY, Winkler JK, Kanalas JJ. Molecular cloning and nucleotide sequence for the complete coding region of human UMP synthase. *Proc Natl Acad Sci USA* 1988; **85**: 1754.

7. Rogers LE, Warford LR, Patterson RB, Porter FS. Hereditary orotic aciduria: I. A new case with family studies. *Pediatrics* 1968; **42**: 415.
8. Haggard ME, Lockhart LH. Megaloblastic anemia and orotic aciduria. A hereditary disorder of pyrimidine metabolism responsive to uridine. *Am J Dis Child* 1967; **113**: 733.
9. Tubergen DG, Krooth RS, Heyn RM. Hereditary orotic aciduria with normal growth and development. *Am J Dis Child* 1969; **118**: 864.
10. Soutter GB, Yu J, Lovric A, Stapleton T. Hereditary orotic aciduria. *Aust Pediatr J* 1970; **6**: 47.
11. Haggard ME, Lockhart LH. Hereditary orotic aciduria, a disorder of pyrimidine metabolism responsive to uridine therapy. *J Pediatr* 1965; **67**: 906.
12. Girot R, Hamet M, Perignon JL *et al*. Cellular immune deficiency in two siblings with hereditary orotic aciduria. *N Engl J Med* 1983; **308**: 700.
13. Fairbanks L, Marinaki AM, Besley GTN. A point mutation resulting in hereditary orotic aciduria with neurological deficits but no megaloblastic anemia. *8th Symposium European Study of Purine and Pyrimidine Metabolism in Man*, 2001: 59.
14. Krooth RS. Properties of diploid cell strains developed from patients with an inherited abnormality of uridine biosynthesis. *Cold Spring Harb Symp Quant Biol* 1964; **29**: 189.
15. McClard RW, Black MJ, Livingstone LR, Jones ME. Isolation and initial characterization of the single polypeptide that synthesizes uridine 5'-monophosphate from orotate in Ehrlich ascites carcinoma. Purification by tandem affinity chromatography of uridine-5'-monophosphate synthase. *Biochemistry* 1980; **19**: 4699.
16. Qumsiyeh MB, Valentine MB, Suttle DP. Localization of the gene for uridine monophosphate synthase to human chromosome region 3q13 by in situ hybridization. *Genomics* 1989; **5**: 160.
17. Suchi M, Mizuno H, Kawai Y *et al*. Molecular cloning of the human UMP synthase gene and characterization of point mutations in two hereditary orotic aciduria families. *Am J Hum Genet* 1997; **60**: 525.
18. Sumi S, Suchi M, Kidouchi K *et al*. Pyrimidine metabolism in hereditary orotic aciduria. *J Inherit Metab Dis* 1997; **20**: 104.
19. McClard R, Black M, Jones M *et al*. Neonatal diagnosis of orotic aciduria: An experience with one family. *J Pediatr* 1983; **102**: 85.
20. Nyhan WL. Nucleotide synthesis via salvage pathway. *Nature*. 2004 Encyclopedia of Life Sciences: 1.
21. van Groeningen CJ, Peters GJ, Nada JC *et al*. Clinical and pharmacologic study of orally administered uridine. *J Natl Cancer Inst* 1991; **83**: 437.
22. Handschumacher RE, Calabresi P, Welch AD *et al*. Summary of current information on 6-azauridine. *Cancer Chemother Rep* 1962; **21**: 1.
23. Pinsky L, Krooth RS. Studies on the control of pyrimidine biosynthesis in human diploid cell strains. II. Effects of 5-azaorotic acid, barbituric acid, and pyrimidine precursors on cellular phenotype. *Proc Natl Acad Sci USA* 1967; **57**: 1267.

DISORDERS OF TRANSPORT AND MINERAL METABOLISM

70.	Cystinuria	525
71.	Cystinosis	532
72.	Hartnup disease	540
73.	Histidinuria	544
74.	Menkes disease	546

Cystinuria

Introduction	525	Treatment	528
Clinical abnormalities	526	References	529
Genetics and pathogenesis	527		

MAJOR PHENOTYPIC EXPRESSION

Calculi in the urinary tract leading to colic or infection, urinary excretion of large amounts of cystine, lysine, ornithine, and arginine, and impaired intestinal absorption of these amino acids.

INTRODUCTION

Cystine (Figure 70.1) owes its name to the fact that it was first recovered from stones obtained from the urinary bladder [1, 2]. The stones of the earliest known cystinuric patients were described by Wollaston in 1810 [1]. He called the material cystic oxide to reflect the origin in the bladder, but Berzelius recognized that the compound in the stones was an amine not an oxide and named it cystine [2]. Its chemical nature was delineated in 1902 by Friedman [3]. Garrod in 1908 discussed cystinuria in the famous Croonian lectures, as being among the original inborn errors of metabolism [4]. Today, we continue to consider that aberrations in transepithelial transport are among the disorders of metabolism.

The renal transport of cystine and the dibasic amino acids has been clarified to a considerable extent by studies over the years on patients with cystinuria. The facts that these patients excrete cystine, ornithine, lysine, and arginine [5–8] and that the plasma concentrations are not increased indicated that they must share a common transport mechanism – one that is defective in cystinuria. That there are similarities in structure (Figure 70.2) makes this intuitively reasonable. It is now clear from studies on

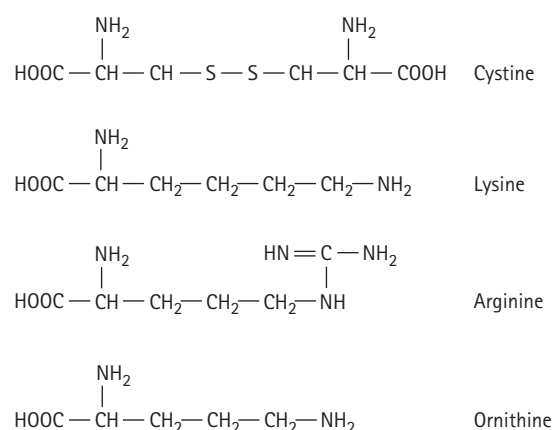


Figure 70.2 Structures of cystine and the dibasic amino acids whose transport is defective in cystinuria.

rat renal tubular fragments and isolated brush-border membrane vesicles that there are two transport systems for cystine: one with a high affinity, low K_m that is shared with the dibasic amino acids [9, 10], and a low affinity system that is not shared. These observations are consistent with physiological studies that had been carried out *in vivo* in cystinuric patients by Dent and Rose [11] who first formulated the idea of a common transport mechanism, shared by the four amino acids, that was defective in cystinuria.

Cystinuria has been divided into at least two types based upon amino acid excretion patterns: type I which is fully recessive and displays normal amino acid excretion in

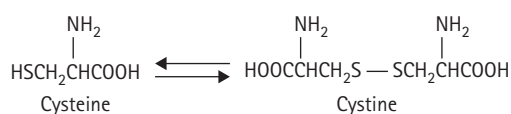


Figure 70.1 Structure of cystine and its relationship to cysteine.

heterozygotes; and an incomplete recessive form in which heterozygotes excrete the relevant amino acids at levels between normal and homozygotes (types II and III) [12].

Patients with cystinuria are normal aside from problems with urolithiasis. There is a distinct disorder which includes cystinuria and severe somatic and developmental delay, identified as hypotonia-cystinuria syndrome [13].

CLINICAL ABNORMALITIES

Cystinuria is of clinical significance because of the insolubility of cystine. The excretion of the dibasic amino acids is without clinical consequences, but cystine is so insoluble that the formation of stones in the urinary tract of cystinuric patients is the rule rather than the exception. Calculi may develop in infancy or childhood (Figures 70.3 and 70.4) [14]. They are usually present before the age of 30 years and they may vary in size from tiny sands or gravel to large staghorn calculi in the renal pelvis or huge calculi in the bladder. They may induce colic or urinary tract obstruction and may require surgical removal. Repeated urinary tract infections can be expected in any patient with renal stone disease. Physical examination may reveal flank tenderness. All of these problems may ultimately lead to renal failure. Some patients have hypertension. Cystine is radio-opaque (Figure 70.4); therefore, the stones can usually be recognized roentgenographically without the



Figure 70.3 CM: A girl with cystinuria, also had dermatomyositis; a disease as common as cystinuria is often found in conjunction with other unrelated conditions.



Figure 70.4 Roentgenogram of the abdomen of CM, illustrating radioopaque renal calculi.



Figure 70.5 J family. Illustrated is the hand of a woman who had cystinuria. She and a number of her family also had Marfan syndrome, another instance of two unrelated diseases, but in this instance they were referred because a positive cyanide-nitroprusside test had led to a diagnosis of homocystinuria.

use of contrast [15, 16]. They can also be demonstrated by ultrasound. Cystine stones themselves are usually yellow-brown in color and have a maple-sugar crystal appearance to the surface. They may contain secondary deposits of calcium, especially following infection.

Twenty-five to 30 percent of patients have evidence of calculi in the first ten years of life [17], 30–35 percent in the next ten years, and some first encounter symptomatic

stone disease in adulthood. Some patients with cystinuria are asymptomatic even as adults (Figure 70.5).

Cystine stones represent 6–8 percent of urinary tract stones of childhood [18], and 1–3 percent of those found in adulthood [19].

The hypotonia-cystinuria syndrome was first identified in an extended Bedouin family [13]. Patients were born with normal growth parameters, but had severe failure to thrive, moderate to severe impaired mental development, dysmorphic facies including frontal bossing, almond-shaped eyes, long eyelashes, depressed nasal bridge, and large, posteriorly rotated ears. Renal and/or bladder cystine calculi were detected in all patients as early as nine months. Jaeken *et al.* [20] reported 11 patients from seven Flemish and two French families who had somewhat milder phenotypes.

GENETICS AND PATHOGENESIS

Cystinuria is transmitted in an autosomal recessive fashion. It is a relatively common inherited disease and neonatal screening programs have yielded prevalence figures from one in 2000 to one in 15,000 [21–23] with a consensus figure of one in 7000 [24]. Both sexes are equally affected, but there is a tendency for more severe disease in males.

Cystine has been recognized by the visualization of the typical hexagonal crystals in microscopic examination of the urinary sediment. However, it has been our experience that uric acid crystals are often mistakenly identified as those of cystine [25]. Many cystinuric individuals are first identified using the cyanide nitroprusside test (Figure 70.6) [26]. The red color obtained is also found in patients with homocystine or other sulfur-containing amino acids in the urine. Confirmation of the amino acid being excreted has been obtained using high voltage electrophoresis, or chromatography on paper or thin layer plates, but the excretion of amino acids should always be quantified. Quantitative amino acid analysis defines the nature of the multiple amino aciduria in cystinuria, and also makes

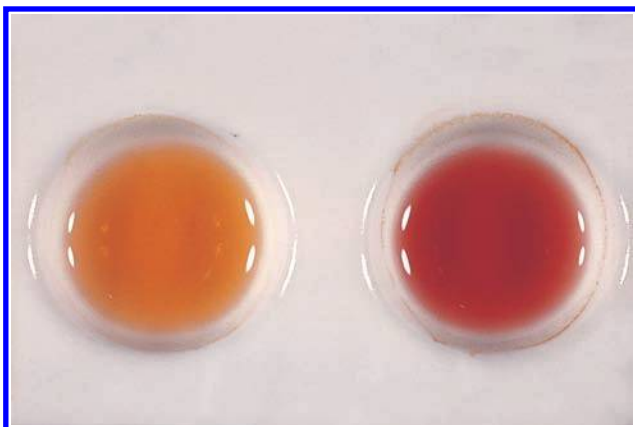


Figure 70.6 Cyanide-nitroprusside test. The red color on the right indicates the presence of sulfur-containing amino acids.

possible the distinction (not always easy) of homozygote from heterozygote and provides prognostic information on the likelihood of the formation of calculi.

Normal individuals excrete cystine, lysine, arginine, and ornithine in amounts less than 10, 60, 5, and 5 mmol/mol creatinine (18, 130, 16, and 22 mg/g creatinine), respectively. Cystinuric individuals have been variously defined as those who excrete more than 120–200 mmol cystine/mol of creatinine (250–400 mg/g creatinine). A patient may be expected to form calculi if the concentrations of cystine in urine regularly exceed 1250 mmol/L (300 mg/L).

In order to separate heterozygotes from homozygotes, Crawhall [27, 28] developed data for 24 stone-forming subjects in 12 different families on the excretion of cystine, lysine, ornithine, and arginine into a canonical variant analysis that has been put into a computer program that generally distinguishes these two populations, but there is overlap between the normal and heterozygous populations. Slow rates of development of renal malabsorption may make the distinction of heterozygotes from homozygotes particularly less reliable in infancy.

The cystinuric pattern of amino aciduria is characterized also by a marked increase in the excretion of lysine and somewhat less of ornithine and arginine [8, 11]. The plasma concentrations of these amino acids are normal. That they are not usually low probably reflects the fact that intestinal absorption of the amino acids of ingested proteins normally proceeds via small peptide absorption, and this is normal in cystinuria [29]. The cystinuric pattern of urinary excretion has also been observed in infants with organic acidemias, such as propionic acidemia, methylmalonic acidemia, and isovaleric acidemia [30], often reverting to normal with metabolic control and reduced excretion of organic acids.

The renal clearance of the dibasic amino acids is increased in cystinuria to a rate usually equal to or somewhat less than the glomerular filtration rate, but in some patients it has exceeded the clearance of inulin [31–33]. The clearance of lysine is usually 50–70 percent of the glomerular filtration rate, and the abnormalities in ornithine and arginine reabsorption are usually less than that of lysine [34]. The excretion of lysine exceeds that of cystine, and the excretion of arginine and ornithine may exceed it as well.

Increasing the filtered load of one of the four amino acids by, for instance, infusion of lysine reduces the reabsorption and increases the clearance of the others in normal individuals and in patients with cystinuria [34–36], although increased cystine excretion may sometimes not occur after a lysine load in a cystinuric patient, presumably because it is already being excreted maximally.

Some other amino acids may be excreted in large amounts in cystinuria; these include glycine [30], cystathionine [37], methionine [38]. The cysteine-homocysteine disulfide [39] is found regularly. Homoarginine [40] and citrulline [41] have also been found in the urine of cystinuric patients.

Studies *in vitro* with isolated tubules and brush-border

membrane vesicles clarified the nature of renal transport defect in cystinuria. There are two renal tubular transport systems for cystine [9, 10, 42]. A high K_m system is not shared by the dibasic amino acids. It is the low K_m shared system that is defective in cystinuria.

Defective intestinal transport has also been demonstrated in cystinuria [43, 44]. Evidence for defective absorption of cystine and the dibasic amino acids was obtained *in vivo* [7, 43–45] by oral loading tests [46]. Intestinal absorption of cysteine is not impaired in cystinuria [47].

Accumulation of basic amino acids in the intestine leads to bacterial decarboxylation to form the diamines, cadaverine, agmatine, and putrescine, which are then absorbed and excreted in the urine. The heterocyclic amine piperidine is also formed from lysine, and pyrrolidine from arginine and ornithine, and these compounds may also be excreted in the urine.

Defective intestinal amino acid transport was also demonstrated in biopsied jejunal mucosa [48–51]. Evidence for heterogeneity was obtained in these studies and among the cystinuric patients, three types were identified [52]: in type I, there was no accumulation of cystine or the dibasic amino acids against a gradient; in type II cystine accumulated, but other dibasic amino acids did not; and in type III, there was accumulation of cystine and the dibasic amino acids to a limited degree. Family studies suggested that some patients classified as type III might be compounds of I and II. These types could also be identified by analysis of amino acid excretion in patients and parents.

The first evidence of heterogeneity in cystinuria came with approaches to carrier detection. It was found that parents of cystinuric patients could be divided into two groups: those who excreted increased amounts of cystine and lysine and those who did not [53]. Cystinuric homozygotes have been classified into three (apparently allelic) groups [54] on the basis of *in vitro* studies of intestinal transport. The urine was normal in the families of type I patients, in whom *in vitro* transport of cystine and lysine was absent, while cystinuria-lysinuria was found in families of patients of types II and III. The quantities of amino acid excreted tended to be larger in type II than in type III heterozygotes [12, 55], but it was evident early that there were a number of compounds [12, 31], and types II and III may be heterogeneous in intestinal absorption. The excretion of arginine was found to be greater in type II than in type III heterozygotes [12]. Elucidation of the nature of mutation has permitted more definitive classification, but some of the observed heterogeneity remains unexplained.

High-affinity, sodium-independent transport of cystine and neutral and dibasic amino acids is conducted by $b^{0,+}$ amino acid transport system, which is a disulfide-linked heterodimeric complex of the rBAT protein and the $b^{0,+AT}$ protein [56]. The rBAT protein, named for the locus of a gene from rabbit renal cortex [57], was mapped to chromosome 2p16.3-21 [58–60]. The product, classified

as SLC3A1, is a type II membrane glycoprotein. Mutations in rBAT were found to result in type I cystinuria [61]. The $b^{0,+AT}$ protein is classified as SLC7A9 (chromosome 19q13.11) [62, 63]; mutations in SLC7A9 were found to cause non-type I cystinuria [59].

An alternate system [64] classifies patients genetically. Those with two biallelic mutations in SLC3A1 comprise type A, those with two mutations in SLC7A9 constitute type B, and patients with one mutation in each gene (rare) are classified as type AB. The total amount and the pattern of dibasic amino acid excretion do not vary significantly between type A and type B patients, and there does not appear to be a significant difference in the phenotype or outcome [64]. Heterozygotes of type B as a group do have significantly higher dibasic amino acid excretion than type A heterozygotes, which is expected from the association of mutations in SLC7A9 associated with non-type I kindreds. However, more than 5 percent of SLC7A9 heterozygotes display dibasic amino acid excretion patterns [64] in the range defining type I [12], so SLC7A9 may be found in any category, and the fine points distinguishing phenotypes remain unexplained and of uncertain significance.

There have been more than 125 mutations identified in the human SLC3A1 gene for rBAT [65], which have largely been missense, such as R180Q, M467K, M467T, P615T, T652R, and L678P, but a few stop codons, deletions, frameshift mutations, and splicing errors have been observed [66, 67]. The missense mutations have generally involved important, highly conserved amino acids. Most mutations have occurred only on a single population, but M467T, the most common to date, has been observed in a broad distribution. Expression of M467T in *Xenopus* led to a protein with 20 percent of normal transport activity [59]. The mutation does not interfere with the affinity of the protein for its substrates, but rather with cellular trafficking so that a small proportion arrives at the plasma membrane where transport takes place [68].

An animal model in naturally cystinuric Newfoundland dogs has a stop codon in exon 2 or the canine gene for rBAT [69]. These dogs excrete over 57 mmol of cystine per mole creatinine (normal 6).

The hypotonia-cystinuria syndrome was associated with a variety of deletions in chromosome 2p21 [20]. The common deleted region included the *SLC3A1* gene and the *PREPL* gene, which codes for a prolyl endopeptidase; the cystinuria is attributable to homozygous deletion of *SLC3A1* and the remainder of the symptoms are presumably due to the deletion of *PREPL*.

TREATMENT

The objective of treatment in cystinuria is the prevention of urinary lithiasis. Crystallization of cystine in urine and the formation of calculi can be minimized by dilution in larger urine volumes [70, 71] or by doing something to alter the concentration of cystine, such as converting it

to a more soluble compound. In order to obtain effective dilution, very large amounts of oral fluid are required. It is particularly important for the patient to get up at night to urinate and drink more. In a patient excreting a gram of cystine, a urine volume of 4 liters is necessary to achieve a concentration of 250 mg/L. In practice, few adults and almost no children comply with an effective regimen. Alkalinization of the urine is of no value; promotion of the solubility of cystine requires a urinary pH of 7.6, which is impossible to achieve physiologically.

For these reasons, penicillamine therapy [72,73] brought about a significant advance in management. Penicillamine (β , β -dimethylcystine) forms a mixed disulfide with cystine (Figure 70.7), which is considerably more soluble than cystine. Its oral administration to patients with cystinuria can reduce cystine concentrations in the urine to levels at which stones will not form (Figure 70.8). Doses of 1–2 g/day may be required to keep the excretion of cystine below 200 mg/g creatinine, at which level the formation of calculi should be prevented. In addition, therapy dissolves stones [74,75].

Penicillamine therapy is demanding because there

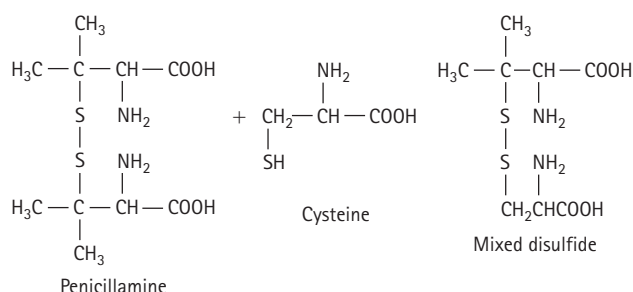


Figure 70.7 Penicillamine and the formation of the cysteine–penicillamine disulfide.

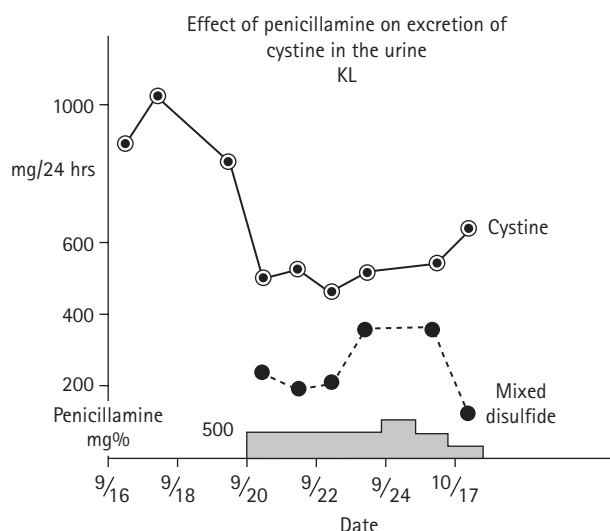


Figure 70.8 Response of a cystinuric patient to the administration of penicillamine.

are many side effects [76–84]. Reactions that have been thought to be allergic occur in as many as half the patients. They are manifested in skin eruptions and fever and some patients develop arthralgias [76]. These reactions are unlike conventional drug allergy in that with continued administration, often after reduced dosage, the reaction will subside, and treatment may then be maintained for many years [84]. More serious side effects, such as nephrosis [77] or pancytopenia, require the withdrawal of the drug. Thrombocytosis has also been observed [79]. A fatal Goodpasture syndrome has been reported in a patient with Wilson disease treated with penicillamine [80]. The compound has effects on collagen and some interesting dermatologic complications have been reported, including epidermolysis [81], pseudoxanthoma elasticum [82], and elastosis perforans serpiginosa [83]. Loss of taste sensation reflects the chelation of copper and is reversed by the administration of copper [84].

N-Acetylpenicillamine is also effective in the formation of mixed disulfides with cystine and appears to cause fewer side effects [85,86]. Mercaptopropionylglycine [87,88] is effective, but it has some of the same side effects as penicillamine. It may be useful in a patient who can no longer tolerate penicillamine. Captopril is a sulfhydryl compound and it has been observed to lower urinary cystine, but to variable degree [89,90].

A variety of interventions may be necessary in patients who develop calculi. Infections should be treated with appropriate antibiotics. Lithotripsy is not as successful in cystinuria as in some other stone-forming diseases. Percutaneous lithotripsy may be effective, or may, by forming smaller units, make surgical lithotomy less formidable. Renal failure may lead to dialysis or renal transplantation [91].

REFERENCES

- Wollaston WH. On cystic oxide: a new species of urinary calculus. *Trans R Soc London* 1810; **100**: 223.
- Berzelius JJ. Calculus urinarius. *Trait Chem* 1833; **7**: 424.
- Friedman E. Der Kreislauf des Schwefels in der Organischen Natur. *Ergebn Physiol* 1902; **1**: 15.
- Garrod AE. Inborn errors of metabolism. *Lancet* 1908; **2**: (lecture I p 2; lecture II p 73; lecture III p 142; lecture IV p 214).
- Yeh HL, Frankl W, Dunn MS *et al*. The urinary excretion of amino acids by a cystinuric subject. *Am J Med Sci* 1947; **214**: 507.
- Stein WH. Excretion of amino acids in cystinuria. *Proc Soc Exp Biol Med* 1951; **78**: 705.
- Dent CE, Senior B, Walshe JM. The pathogenesis of cystinuria II: polarographic studies of the metabolism of sulphur-containing amino acids. *J Clin Invest* 1954; **33**: 1216.
- Arrow VK, Westall RG. Amino acid clearances in cystinuria. *J Physiol* 1958; **142**: 141.
- Foreman JW, Hwang SM, Segal S. Transport interactions of cystine and dibasic amino acids in isolated rat renal tubules. *Metabolism* 1980; **29**: 53.

10. Segal S, McNamara PD, Pepe LM. Transport interaction of cystine and dibasic amino acids in renal brush border vesicles. *Science* 1977; **197**: 169.
11. Dent CE, Rose GA. Amino acid metabolism in cystinuria. *Q J Med* 1951; **20**: 205.
12. Kelly S. Cystinuria genotypes predicted from excretion patterns. *Am J Med Genet* 1978; **2**: 175.
13. Parvari R, Brodyansky I, Elpeleg O *et al*. A recessive contiguous gene deletion of chromosome 2p16 associated with cystinuria and a mitochondrial disease. *Am J Hum Genet* 2001; **69**: 869.
14. Fawcett NP, Nyhan WL. Cystinuria and dermatomyositis. *Clin Pediatr* 1970; **9**: 727.
15. Renander A. The roentgen density of the cystine calculus. *Acta Radiol Suppl* 1941; **41**: 35.
16. Hambræus L, Lagergren C. Cystinuria in Sweden. VI Biophysical and roentgenological studies of urinary calculi from cystinurics. *J Urol* 1962; **88**: 826.
17. Stephens AD. Cystinuria and its treatment: 25 years experience at St Bartholomew's Hospital. *J Inherit Metab Dis* 1989; **12**: 197.
18. Millner DS. Cystinuria. *Endocr Metab Clin N Am* 1990; **19**: 889.
19. Singer A. Cystinuria: a review of the pathophysiology and management. *J Urol* 1989; **142**: 669.
20. Jaeken J, Martens K, Francois I *et al*. Deletion of PREPL, a gene encoding a putative serine oligopeptidase, in patients with hypotonia-cystinuria syndrome. *Am J Hum Genet* 2006; **78**: 38–51.
21. Woolf LI. Large-scale screening for metabolic disease in the newborn in Great Britain. In: Anderson JA, Swaiman KF (eds). *Phenylketonuria and Allied Metabolic Disorders*. Washington DC: US Dept of Health Education and Welfare Children's Bureau, 1967: 50.
22. Turner B, Brown DA. Amino acid excretion in infancy and early childhood: a survey of 200000 infants. *Med J Aust* 1972; **1**: 62.
23. Levy HL, Shih VE, Madigan PM. Massachusetts metabolic disorders screening program. I. Technics and results of urine screening. *Pediatrics* 1971; **49**: 825.
24. Levy HL. Genetic screening. In: Harris H, Hirschhorn K (eds). *Advances in Human Genetics*, vol. 4. New York: Plenum Press, 1973: 1.
25. Lesch M, Nyhan WL. A familial disorder of uric acid metabolism and central nervous system function. *Am J Med* 1964; **36**: 561.
26. Brand E, Harris MM, Biloon S. Cystinuria: excretion of a cystine complex which decomposes in the urine with the liberation of free cystine. *J Biol Chem* 1930; **86**: 315.
27. Crawhall JC, Purkiss P, Watts RWE, Young EP. The excretion of amino acids by cystinuric patients and their relatives. *Ann Hum Genet* 1969; **33**: 149.
28. Crawhall JC. Cystinuria diagnosis and treatment. In: Nyhan WL (ed.). *Heritable Disorders of Amino Acid Metabolism*. New York: Wiley, 1974: 593.
29. Purkiss P, Chalmers RA, Borud O. Combined iminoglycinuria and cystine and dibasic aminoaciduria in patients with propionic acidemia and 3-methylcrotonylglycinuria. *J Inherit Metab Dis* 1980; **3**: 85.
30. Delvalle JA, Merinero B, Garcia MJ *et al*. Biochemical findings in a patient with neonatal methylmalonic acidemia. *J Inherit Metab Dis* 1982; **5**: 53.
31. Morin CL, Thompson MW, Jackson SH, Sass-Kortsak A. Biochemical and genetic studies in cystinuria: observations on double heterozygotes of genotype I/II. *J Clin Invest* 1971; **50**: 1961.
32. Frimpter GW, Horwith M, Furth E *et al*. Inulin and endogenous amino acid renal clearances in cystinuria: evidence for tubular secretion. *J Clin Invest* 1962; **41**: 281.
33. Crawhall JC, Scowen EF, Thompson CJ, Watts RWE. The renal clearance of amino acids in cystinuria. *J Clin Invest* 1967; **46**: 1162.
34. Kato T. Renal handling of dibasic amino acids and cystine in cystinuria. *Clin Sci Mol Med* 1977; **53**: 9.
35. Lester FT, Cusworth DC. Lysine infusion in cystinuria: theoretical renal thresholds for lysine. *Clin Sci* 1973; **44**: 99.
36. Robson EB, Rose GA. The effect of intravenous lysine on the renal clearances of cystine arginine and ornithine in normal subjects in patients with cystinuria and Fanconi syndrome and their relatives. *Clin Sci* 1957; **16**: 75.
37. Frimpter GW. Cystathioninuria in a patient with cystinuria. *Am J Med* 1969; **46**: 832.
38. King JS Jr, Wainer A. Cystinuria with hyperuricemia and methioninuria: biochemical study of a case. *Am J Med* 1967; **43**: 125.
39. Frimpter GW. The disulfide of L-cysteine and L-homocysteine in urine of patients with cystinuria. *J Biol Chem* 1961; **236**: 651.
40. Cox BD, Cameron JC. Homoarginine in cystinuria. *Clin Sci Mol Med* 1974; **46**: 173.
41. Milne MD, London DR, Asatoor AM. Citrullinuria in cases of cystinuria. *Lancet* 1962; **2**: 49.
42. McNamara PD, Pepe LM, Segal S. Cystine uptake by renal brush border vesicles. *Biochem J* 1969; **194**: 443.
43. Milne MD, Asatoor AM, Edwards KDG, Loughridge LW. The intestinal absorption defect in cystinuria. *Gut* 1961; **2**: 323.
44. Asatoor AM, Lacey BW, London DR, Milne MD. Amino acid metabolism in cystinuria. *Clin Sci* 1962; **23**: 285.
45. Rosenberg LE, Durant JL, Holland JM. Intestinal absorption and renal extraction of cystine and cysteine in cystinuria. *N Engl J Med* 1965; **273**: 1239.
46. Brand E, Cahill GF. Further studies on metabolism of sulfur compounds in cystinuria. *Proc Soc Exp Biol Med* 1934; **31**: 1247.
47. Silk DB, Perrett D, Stephens AD *et al*. Intestinal absorption of cystine and cysteine in normal human subjects and patients with cystinuria. *Clin Sci Mol Med* 1974; **47**: 393.
48. Thier S, Fox M, Segal S, Rosenberg LE. Cystinuria: *in vitro* demonstration of an intestinal transport defect. *Science* 1964; **143**: 482.
49. McCarthy CF, Borland JL, Lynch HJ *et al*. Defective uptake of basic amino acids and L-cystine by intestinal mucosa of patients with cystinuria. *J Clin Invest* 1964; **43**: 1518.
50. Thier S, Segal S, Fox M *et al*. Cystinuria: defective intestinal transport of dibasic amino acids and cystine. *J Clin Invest* 1965; **44**: 442.

51. Coicadan L, Heyman M, Grasset E, Desjeux JF. Cystinuria: reduced lysine permeability at the brush border of intestinal membrane cells. *Pediatr Res* 1980; **14**: 109.
52. Goodyer PR, Clow C, Reade T, Girardin C. Prospective analysis and classification of patients with cystinuria identified in a newborn screening program. *J Pediatr* 1993; **122**: 568.
53. Harris H, Mittwoch U, Robson EB, Warren FL. Phenotypes and genotypes in cystinuria. *Ann Hum Genet* 1955; **20**: 57.
54. Rosenberg LE. Genetic heterogeneity in cystinuria. In: Nyhan WL (ed.). *Amino Acid, Metabolism and Genetic Variation*. New York: McGraw-Hill, 1967: 341.
55. Rosenberg LE, Durant JL, Albrecht I. Genetic heterogeneity in cystinuria: evidence for allelism. *Trans Assoc Am Phys* 1966; **79**: 284.
56. Pfeiffer R, Loffing J, Rossier G et al. Luminal heterodimeric amino acid transporter defective in cystinuria. *Mol Biol Cell* 1999; **10**: 4135.
57. Bertran J, Werner A, Moore ML et al. Expression cloning of a cDNA from rabbit kidney cortex that induces a single transport system for cystine and dibasic and neutral amino acid. *Proc Natl Acad Sci USA* 1992; **89**: 5601.
58. Pras E, Arber N, Aksentijevich I et al. Localization of a gene causing cystinuria to chromosome 2p. *Nat Genet* 1994; **6**: 415.
59. Calonge MJ, Gasparini P, Chillaron J et al. Cystinuria caused by mutations in rBAT a gene involved in the transport of cystine. *Nat Genet* 1994; **6**: 420.
60. Wright EM. Cystinuria defect expresses itself. *Nat Genet* 1994; **6**: 328.
61. Pras E, Raben N, Golomb E et al. Mutations in the SLC3A1 transporter gene in cystinuria. *Am J Hum Genet* 1995; **56**: 1297.
62. Bisceglia L, Calonge MJ, Totaro A et al. Localization by linkage analysis of the cystinuria type III gene to chromosome 19q131. *Am J Hum Genet* 1997; **60**: 611.
63. Wartenfeld R, Golomb E, Katz G et al. Exclusion of the SLC3A1 gene and mapping of a new locus on 19q. *Am J Hum Genet* 1997; **60**: 617.
64. Dello Strologo L, Pras E, Pontesilli C et al. Comparison between SLC3A1 and SLC7A9 cystinuria patients and carriers: a need for a new classification. *J Am Soc Nephrol* 2002; **13**: 2547.
65. Stenson PD, Ball EV, Howells K et al. The Human Gene Mutation Database: providing a comprehensive central mutation database for molecular diagnostics and personalized genomics. *Hum Genomics* 2009; **4**: 69.
66. Saadi I, Chen XZ, Hediger M et al. Molecular genetics of cystinuria: mutation analysis of SLC3A1 and evidence for another gene in the type I (silent) phenotype. *Kidney Int* 1998; **54**: 48.
67. Pras E, Golomb E, Drake C et al. A splicing mutation (891 + 4A → G) in SLC3A1 leads to exon 4 skipping and causes cystinuria in a Moslem Arab family. *Hum Mutat* 1998; **1**(Suppl.): S28.
68. Chillaron J, Estévez R, Samarzija I et al. An intracellular trafficking defect in type I cystinuria rBAT mutants M467T and M467K. *J Biol Chem* 1997; **272**: 9543.
69. Casal ML, Giger U, Bovee KC, Patterson DF. Inheritance of cystinuria and renal defect in Newfoundlands. *J Am Vet Med Assoc* 1995; **207**: 1585.
70. Dent CE, Friedmann M, Green H, Watson LCA. Treatment of cystinuria. *Br Med J* 1965; **1**: 403.
71. Dent CE, Senior B. Studies on the treatment of cystinuria. *Br J Urol* 1955; **27**: 317.
72. Crawhall JC, Scowen EF, Watts RWE. Effect of penicillamine on cystinuria. *Br Med J* 1963; **1**: 585.
73. Crawhall JC, Scowen EF, Watts RWE. Further observations on use of d-penicillamine in cystinuria. *Br Med J* 1964; **1**: 1411.
74. McDonald JE, Henneman PH. Stone dissolution *in vivo* and control of cystinuria with d-penicillamine. *N Engl J Med* 1965; **273**: 578.
75. Crawhall JC, Scowen EF, Thompson CJ, Watts RWE. Dissolution of cystine stones during d-penicillamine treatment of a pregnant patient with cystinuria. *Br Med J* 1967; **1**: 216.
76. Bartter FC, Lotz M, Thier S et al. Cystinuria: combined clinical staff conference at the National Institutes of Health. *Ann Intern Med* 1965; **62**: 796.
77. Fellers FX, Shahidi NT. The nephrotic syndrome induced by penicillamine therapy. *Am J Dis Child* 1959; **98**: 669.
78. Corcos JM, Soler-Bechera J, Mayer K et al. Neutrophilic agranulocytosis during administration of penicillamine. *J Am Med Assoc* 1964; **189**: 2654.
79. Fawcett NP, Nyhan WL, Anderson WW. Thrombocytosis during treatment of cystinuria with penicillamine. *J Am Med Assoc* 1968; **203**: 381.
80. Sternlieb I, Bennett B, Scheinberg IH. D-penicillamine-induced Goodpasture's syndrome in Wilson's disease. *Ann Intern Med* 1975; **82**: 673.
81. Beer WE, Cooke KB. Epidermolysis bullosa induced by penicillamine. *Br J Dermatol* 1967; **79**: 123.
82. Bolognia JL, Braverman I. Pseudoxanthoma-elasticum-like skin changes induced by penicillamine. *Dermatology* 1992; **184**: 12.
83. Sahn EE, Maize JC, Garen PD et al. D-penicillamine-induced elastosis perforans serpiginosa in a child with juvenile rheumatoid arthritis. *J Am Acad Dermatol* 1969; **20**: 979.
84. Henkin RI, Keiser HR, Jaffe IA et al. Decreased taste sensitivity after d-penicillamine reversed by copper administration. *Lancet* 1967; **16**: 1268.
85. Stokes GS, Potts JT, Lotz M, Bartter F. A new agent in the treatment of cystinuria: N-acetyl-d-penicillamine. *Br Med J* 1968; **1**: 283.
86. Stephens AD, Watts RWE. The treatment of cystinuria with N-acetyl-penicillamine a comparison with the results of d-penicillamine treatment. *Q J Med* 1971; **40**: 335.
87. King JS. Treatment of cystinuria with alpha-mercapto-propionylglycine: a preliminary report. *Proc Soc Exp Biol Med* 1968; **129**: 927.
88. Kinoshita K, Yachiku S, Kotake T et al. Treatment of cystinuria with alpha-mercaptopyrionylglycine (MPG). *Jap J Clin Med* 1972; **30**: 232.
89. Perezella MA, Bullen GK. Successful treatment of cystinuria with captopril. *Am J Kidney Dis* 1993; **21**: 504.
90. Dahlberg PJ, Jones JD. Cystinuria: failure of captopril to reduce cystine excretion. *Arch Intern Med* 1989; **149**: 713.
91. Kelly S, Nolan DP. Letter to the editor. *J Am Med Assoc* 1980; **243**: 1897.

Cystinosis

Introduction	532	Treatment	536
Clinical abnormalities	533	References	537
Genetics and pathogenesis	535		

MAJOR PHENOTYPIC EXPRESSION

Nephropathy progressive to renal failure; a Fanconi syndrome of glycosuria, phosphaturia, and generalized amino aciduria leading to acidosis and rickets; inhibition of growth; fair skin, hair, and irides; cystine crystals in bone marrow and other tissues; increased cellular lysosomal cystine; and defective adenosine triphosphate (ATP)-dependent lysosomal carrier mediated efflux of cystine resulting from abnormalities in cystinosin, a transmembrane lysosomal transporter.

INTRODUCTION

Cystinosis was first described in 1903 by Abderhalden [1] in a report of a patient in whose tissue deposits were identified chemically as cystine. In 1924, cystine deposits were also documented in the report of Lignac [2] of three infants with rickets, shortness of stature, and renal disease. In 1931, Fanconi [3] reported a child with rickets, dwarfism, and glycosuria, and this picture of renal tubular defect has come to be known as the Fanconi syndrome, or the renal Fanconi syndrome to distinguish it from Fanconi anemia. A patient with renal dwarfism, vitamin D-resistant rickets, spontaneous fractures, hypophosphatemia, acidosis, and glycosuria was reported by de Toni [4] in 1933, and a similar patient was reported by Debré and colleagues [5] in 1934. In 1936, Fanconi [6] proposed that all of these patients constituted a syndrome of dwarfism and renal defect with glycosuria and hypophosphatemic rickets. The syndrome has been referred to as that of Lignac, Fanconi, De Toni, and Debré, in various orders. The amino aciduria was documented by Dent [7]. This renal tubular abnormality may be seen in other conditions besides cystinosis. Its clear association with cystinosis was made by Fanconi and Bickel [8] and Bickel *et al.* [9].

Schneider and colleagues [10, 11] first characterized patients with cystinosis as having increased quantities of cystine in leukocytes and cultured fibroblasts. This provided a highly specific method of diagnosis. The lysosomal localization of the stored cystine was reported

on the basis of electron microscopy by Patrick and Lake [12] and by Harms and Schneider on the basis of chemical fractionation [13]. The abnormality in cystine transport was discovered independently in 1982 by Steinherz and colleagues [14] and by Jonas and colleagues [15]. Isolated lysosomes from leukocytes or cultured lymphoblasts or fibroblasts of patients were also shown by Gahl and colleagues [16] and by Jonas and colleagues [17] to have defective transport of cystine out of the organelle. The

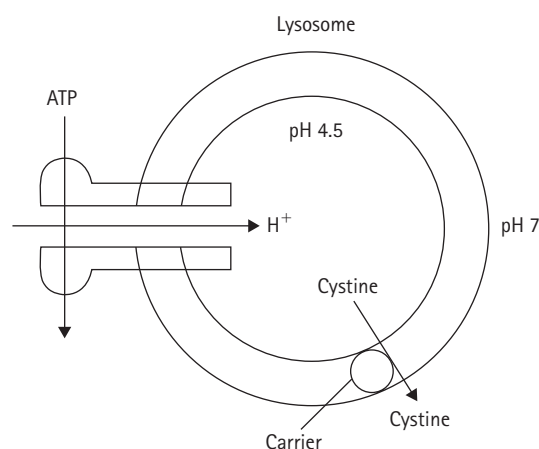


Figure 71.1 Postulated lysosomal site of the defect in cystinosis in a cystine carrier (reprinted with permission from Nyhan WL. *Abnormalities in Amino Acid Metabolism in Clinical Medicine*. Norwalk, CT: Appleton Century Crofts, 1984).

efflux which is defective in cystinosis is linked to ATP, in a process in which a specific ATPase acts as a proton pump (Figure 71.1). This has been the prototype for other lysosomal transporters that are involved in human disease, such as those for cobalamin (Chapter 4) and sialic acid.

CLINICAL ABNORMALITIES

In classic cystinosis, the patient appears normal at birth. The usual presentation is at six to ten months of age with symptoms generated by renal tubular dysfunction. These patients have a classic renal Fanconi syndrome (Table 71.1) in which there is renal tubular deficiency in the reabsorption of glucose, phosphate, amino acids, and other organic acids, as well as of sodium, potassium, calcium, magnesium, bicarbonate, and water [18–21]. The aminoaciduria is generalized, with those amino acids usually found in the urine in moderate quantity, such as glycine, excreted in very large amounts; those usually found in very small amounts, such as leucine, excreted in moderate amounts; and the imino acids, such as proline, which are not normally found in urine, excreted in appreciable amounts.

Renal losses of phosphate lead to hypophosphatemia and rickets. Any of the clinical manifestations of rickets may develop, and roentgenograms reveal the typical cupped and frayed ends of the long bones. A thoracic rosary is characteristic. Alkaline phosphatase levels are increased. This is a form of vitamin D-resistant rickets in the sense that it develops in spite of the usual antirachitic doses of vitamin D, and does not respond to vitamin D in these amounts. Losses of sodium bicarbonate and potassium lead to chronic acidosis and hypokalemia. This is a proximal renal tubular acidosis. It is hyperchloremic and a low serum bicarbonate is consistent with very great losses of bicarbonate. In normal individuals, the sum of the urinary concentrations of sodium and potassium is lower than that of chloride (a negative urinary anion gap). In renal tubular acidosis, their concentration is higher than that of chloride (positive urinary anion gap).

Impaired growth may be extreme. Patients may have tremendous polyuria and polydipsia. This makes these children highly vulnerable to dehydration, especially in

the presence of otherwise minor infections, particularly gastrointestinal disease that interferes with fluid intake. In a patient in whom continued administration of large amounts of sodium bicarbonate is maintained, hypernatremic dehydration may occur. Symptomatic hypokalemia may present with recurrent episodes of weakness or prostration. Cardiovascular collapse and sudden death may occur. The patient with cystinosis may present initially with the picture of the Bartter syndrome [22–24]. There is proteinuria particularly of lower molecular weight proteins (tubular proteinuria) [25], and protein excretion may reach 5 g a day. The renal tubular defect may lead to very great losses of free-carnitine [26], which is normally about 97 percent reabsorbed. Low levels of free-carnitine in the blood and muscle lead to deficient fatty acid oxidation, microvesicular fat in muscle and liver, and clinical myopathy. Urinary tract calculi, of both urate and calcium oxalate, have been reported [27]. The increased excretion of calcium may lead to nephrocalcinosis [28, 29].

Glomerular involvement occurs later than renal tubular defects, but creatinine clearance decreases over time. Uremia and renal failure are progressive, leading usually to death within the first ten years of life if not treated. The mean age of renal death in a 1984 study of 205 patients in Europe was 9.2 years [30]. The report of renal survival in 2010 [31] demonstrates the effect of cysteamine, with the majority of patients who began treatment at <2.5 years having normal GFR at ten years of age; four of nine with early treatment followed for more than ten years had



Figure 71.2 WB: A seven-year-old boy with advanced cystinosis had the characteristic very blond hair and pot belly. His height was 108 cm. (The illustration was kindly provided by Dr Jerry Schneider of the University of California San Diego.)

Table 71.1 Differential diagnosis of the renal Fanconi syndrome

Cystinosis
Idiopathic
Hepatorenal tyrosinemia
Galactosemia
Wilson disease
Lowe syndrome
Glycogen storage disease (I, III)
Lysinuric protein intolerance



Figure 71.3 RD: A petite blond Arab girl with cystinosis. She was four years old and 83.6 cm tall (50th percentile for 21 months). Her weight was 12 kg. Cystine crystals were identified on skin biopsy and slit lamp examination of the cornea revealed refractile bodies characteristic of cystinosis.

serum creatinine >2.5 mg/dL, compared to 12 of 12 with later initiation of treatment.

Shortness of stature is typical in cystinosis. The process begins in the first year. By eight years, the height is at the 50th percentile for four years [32]. Bone age may be delayed [33]. Height and weight are similarly delayed; so the patient appears proportionate. Sparing of brain growth may give an appearance of relative macrocephaly.

Patients generally have a fair complexion and very fair hair (Figure 71.2). However, a dark skin color is not against the diagnosis. The disease has been seen in several non-Caucasian patients (Figure 71.3) including Black children [34, 35]. Ability to sweat may be impaired [36] and this may lead to intolerance to heat. There may also be impaired production of tears and saliva.

A variety of ophthalmic abnormalities are seen in patients with cystinosis. Refractile crystalline bodies may be demonstrated by slit lamp examination of the cornea [37]. These birefringent refractile bodies are pathognomonic of cystinosis. The corneal deposits are not present at birth, but they may be found well before clinical nephropathy [38]. Ultimately, there may be a thickened cornea with a distinct haziness [39, 40]. Corneal ulcers may occur [41], and if untreated, the changes may proceed from superficial punctuate keratopathy to filamentary keratopathy, severe peripheral neovascularization, and band keratopathy with posterior synechiae with iris thickening in older patients [42]. Crystalline cystine has been identified in the conjunctiva by x-ray diffraction, as well as by biopsy

[43], and the crystal density can be followed with *in vivo* confocal microscopy [44]. Photophobia is a prominent and disturbing symptom. Patients with cystinosis also have a characteristic retinopathy [45]. The peripheral retina has a patchy pattern of hyperpigmentation and depigmentation, often in regular distribution, varying from about a tenth of a disk diameter to a fine, peppery size. Retinal changes, which are usually more marked temporally, may be the earliest clinical manifestation of the disease. The ability to keep patients alive longer with renal transplantation has permitted the development of much more marked ocular changes. Visual acuity is not usually impaired in children, but among those treated with renal transplantation and living into the third decade, some can be legally blind.

Another late complication is hypothyroidism [46–48]. Atrophy of the gland results from cystine accumulation, and more than 70 percent of patients over ten years of age need replacement therapy [49]. Thyroid stimulating hormone (TSH) may be elevated before the development of clinical hypothyroidism [50]. Some patients develop insulin-dependent diabetes mellitus [51] or exocrine pancreatic insufficiency [52]. Hypogonadism is common in adult males [53]. Azoospermia seems to be a universal finding [54], although there may be evidence of spermatogenesis in some cases. Puberty may be delayed in the female, but is ultimately normal, and there have been normal pregnancy outcomes [55, 56]. Myopathy results from crystalline deposition in muscle, and this may be manifest in visible atrophy of muscles [57]. Symptoms may be aggravated by carnitine deficiency.

Impaired visual and spatial cognition has been recognized in cystinosis [58], but function is normal as is intelligence. There also may be late involvement of the central nervous system [59, 60], or cerebral atrophy visible on neuroimaging [59]. In a study of magnetic resonance imaging (MRI) from 49 patients [61], 54 percent were read as normal, 24 percent had mild volume loss, 11 percent had severe volume loss, and 11 percent had isolated Chiari I malformation. Some have had seizures, tremor, pyramidal signs, or impaired mental development [62, 63]. Gait disturbance made one patient wheelchair-bound, but neurological examination is usually normal [63]. A study of adequately treated children, aged two to 17 years, documented difficulties in fine-motor coordination which appeared in early childhood, but were nonprogressive and not related to brain structural changes [61].

Clinical heterogeneity has been defined clinically in cystinosis. A number of variant phenotypes has been described. At the other extreme from classic nephropathic cystinosis is a benign or so-called adult variant in which patients develop neither nephropathy nor retinal symptomatology [64, 65]. These patients have been identified by the presence of crystalline deposits in the cornea, bone marrow, and leukocytes. Kidney biopsies, however, demonstrated no cystine crystals and the cystine content of the kidney was not elevated [65, 66]. They may

develop photophobia in middle age, but it is usually not severe. They do not have retinopathy.

Other patients have been described with intermediate phenotypes [67–69]. These patients all have renal abnormalities, but they do not present until an older age. The Fanconi syndrome may be less than complete, and the glomerular disease may progress slowly. Among involved siblings in a family, the phenotype tends to be quite similar. Overall, it is likely that there are a number of different variants. Photophobia and retinopathy are variable. Some patients have had some delay in growth, while others have grown normally.

Abnormal laboratory tests observed in patients with cystinosis include elevated sedimentation rates, platelet counts, and cholesterol [33]. With the advent of uremia, the patient may become anemic.

The pathology of the disease reflects the extensive deposition of cystine crystals throughout the tissues of the body. The disorder may be readily diagnosed by the examination of aspirated bone marrow for cystine crystals (Figure 71.4). Conjunctival biopsy has also been employed for this purpose. Crystals may also be demonstrated in the lymph nodes. Its deposition in the kidney leads to the nephropathy that characterizes the disease. This has the appearance of chronic interstitial nephritis, with glomerular endothelial proliferation, hyalinization, and necrosis.

GENETICS AND PATHOGENESIS

Cystinosis is transmitted by an autosomal recessive gene. Each of the forms of cystinosis follows this pattern of inheritance. The incidence of the disease in North America is one per 100,000–200,000 births, but in France the incidence in Brittany is one in 26,000 [70].

The chemical hallmark of this disorder is the accumulation of large amounts of cystine in cells [10, 11]. The measurement of cystine content in freshly isolated

leukocytes or in cultured fibroblasts is diagnostic. The values in nephropathic cystinosis are 80–100 times the control value. In benign cystinosis, levels 30 times normal are found, and in the intermediate group the values tend to be intermediate, although there are exceptions [10, 67, 71]. Within the cell, the cystine is stored in the lysosomes [12, 13].

Studies of cystine efflux from lysosomes have clarified the fundamental defect in cystinosis. When cultured cells are treated with cysteine-glutathione mixed disulfide or cystine dimethylester so that they accumulate large amounts of cystine, normal cells lose cystine progressively over 20–90 minutes, but cystinotic cells do not [14, 15]. Lysosomes from freshly isolated normal leukocytes behaved *in vitro* like normal intact cells. They lose cystine progressively, while those isolated from cystinotic leukocytes do not [16]. There is a carrier-mediated lysosomal transport system for cystine, and it is defective in cystinosis. The process is energy related; the addition of 2,4-dinitrophenol to deplete normal cells of ATP inhibited the efflux of cystine. When ATP was added to lysosomes isolated from normal lysosomal fibroblasts, there was rapid, progressive efflux of cystine [17]. ATP had no effect on efflux from the lysosomes of cystinotic cells. Thus, it is an ATP-linked efflux of cystine that is aberrant in cystinosis (Figure 71.1). In cystinosis, the ATPase was found to be normal as was lysosomal proton translocating activity [72], indicating that the defect was in the cystine carrier protein itself.

Heterozygotes may be detected by determining the cystine content of leukocytes or fibroblasts [10, 11, 71]. In nephropathic cystinosis, the cells of heterozygotes display a mean cystine content five to six times the normal mean. Although these data have been obtained on both fibroblasts and leukocytes, the leukocyte assay allows for noninvasive testing and recurring monitoring. The clearance of cystine from leukocytes loaded with 35S-cystine dimethylester has been developed for the detection of heterozygosity [73]. Cystine efflux from isolated leukocyte lysosomes occurs at about half the normal maximal rate in heterozygotes [16].

Prenatal diagnosis on the basis of the content of cystine in cultured amniocytes or chorionic villus cells is a well-established technique [74–76].

The gene for cystinosis, *CTNS*, codes for the transporter protein cystinosin [18, 77–79]. It contains 12 exons and spans over 23 kb of genomic DNA [78]. The gene for cystinosin was mapped to chromosome 7p13 in 1995 [77]. A number of mutations have been identified [18, 78, 79].

The protein contains 367 amino acids and has seven transmembrane domains and seven sites of potential glycosylation. It transports cystine and has appropriate kinetic properties [80].

More than 85 mutations have been identified [18, 81]. The most common, found in 50 percent of nephropathic cystinosis patients from northern Europe, is a 57-kb deletion that removes the first ten exons and is readily detectable by a multiplex polymerase chain reaction assay [78, 79, 82–84]. The mutation deletes expression of the

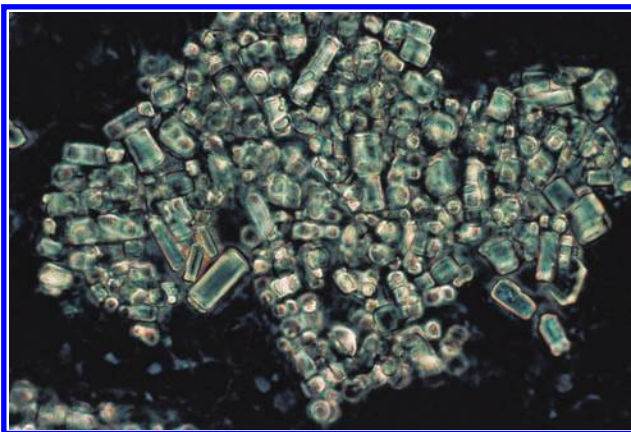


Figure 71.4 Cystine crystals aspirated from the marrow of a patient with cystinosis. (The illustration was kindly provided by Dr Jerry Schneider of the University of California San Diego.)

protein and displays a founder effect dating from 500AD in Germany. The second most common nephropathic mutation, observed in 12 percent of American patients, was a G753A, W138X stop mutation [18, 78]. Mutations have been found in the promoter of the *CTNS* gene [85], including a $-295G \rightarrow C$ substitution in a patient with nephropathic cystinosis and two changes at -303 , a T insertion and a $G \rightarrow T$ transition in two patients with ocular cystinosis. Some correlations of genotype and phenotype have been possible [85]. Mutational analysis may be used effectively in heterozygote detection and in prenatal diagnosis.

TREATMENT

Early diagnosis is a prerequisite for successful therapy. Glomerular damage, once developed, is not reversible.

Supportive therapy in nephropathic cystinosis is demanding. The renal tubular defect requires the provision of adequate amounts of water, sodium, and potassium. Unrestricted access to salt and water is important. Polycitra, which contains 1 mEq of sodium and 1 mEq of potassium per milliliter, is convenient for electrolyte replacement [21]. In some patients whose potassium is normal, Bicitra (which contains only sodium citrate) or sodium bicarbonate may be used. The average dose is 45–60 mL/day. Rickets is treated with 10,000–15,000 units of vitamin D per day, or an equivalent amount of 1,25-dihydrocholecalciferol. Phosphate replacement permits reduction of the dose of vitamin D. In fact, healing of rickets may be obtained in this disorder using phosphate alone.

In the presence of intercurrent illness in which there is vomiting or diarrhea, a vigorous approach to parenteral fluid therapy is important. A patient with renal tubular acidosis receiving polycitra or sodium bicarbonate may present markedly dehydrated, but with a high concentration of sodium in the blood. It is important not to treat this volume-depleted patient as if nondehydrated sodium-intoxicated, gingerly using an administration of nonsodium-containing solutions calculated to lower the sodium gradually. Such an approach can readily lead to irreversible oligemic shock.

Many patients with nephropathic cystinosis have been treated with renal transplantation [49, 86]. Following transplantation, growth does not usually improve, and visual disease and thyroid disease tend to progress. Nevertheless, it is clear that nephropathy does not develop in the transplanted kidney, and many patients have lived 20 or more years post-transplant.

A variety of approaches has been undertaken to treat cystinosis using thiol compounds. Intracellular cystine can be lowered in cultured cells using a number of compounds. The most effective thiol compound is cysteamine (Figure 71.5) [87]. This aminothiol compound with its neutral amino group readily enters the lysosome, where it reacts with cysteine to form the mixed disulfide, whose structure

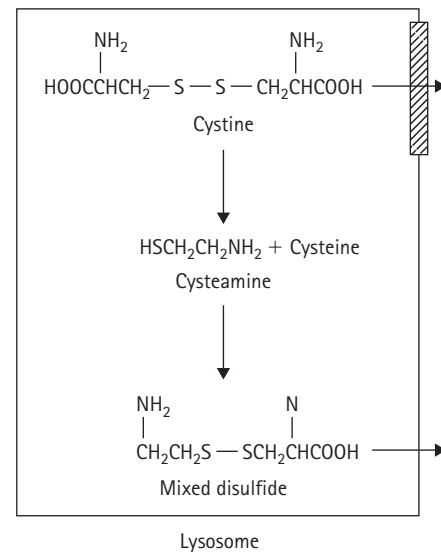


Figure 71.5 The structure of cysteamine and its mechanism for depletion of stored cystine.



Figure 71.6 WG: An 18-year-old with cystinosis illustrating the efficiency of treatment with cysteamine since diagnosis at about one year of age. She has not had a renal transplantation and has continued to do well.

is sufficiently similar to lysine to be transported out via the lysine transporter. Doses approximating 1.3 g/m^2 ($50\text{--}90 \text{ mg/kg}$) per day have now been used for many years (Figure 71.6) with rewarding results [32, 88]. The starting dose is usually 10 mg/kg per day and increased by 10 mg/kg per day until the target is reached. Adults may start at 50 mg/day , increasing every few days as tolerated to a target of 1.3 g/m^2 per day. Depletion of stored cystine can

be documented by analysis of leukocytes to be as much as 80 percent. Growth of treated children was 50–60 percent of the normal rate.

Renal tubular dysfunction, once established, does not improve, and it still develops with cystamine treatment, but its severity may be reduced. Glomerular function has been remarkably improved [89], particularly with early treatment, although there is still inexorable decline over years. Greco and colleagues [31] reported a change of GFR of -0.27 ± 0.91 mL/min/1.73 m² per year in cysteamine-treated patients, and $+0.06 \pm 0.78$ mL/min/1.73 m² per year in treated patients. Cysteamine will protect the thyroid, obviating the need for thyroxine replacement therapy [90]. The taste and smell of cysteamine are quite disagreeable. Capsules of cysteamine bitartrate (Cystagon, Mylan) are preferred by those who can swallow capsules. Gastrointestinal symptoms are virtually the rule in patients treated with cysteamine; they are acid-related and improve with treatment with omeprazole (10–20 mg twice a day) [91]. A derivative, phosphocysteamine, is equally effective *in vitro* [92] and, as it is both odorless and tasteless, it has advantages for human use. The two compounds can be used interchangeably. Neither is effective orally in managing the corneal disease, but cysteamine eye drops are remarkably effective if used 10–14 times a day [93]. Absorption of cysteamine in the small intestine is more effective and better tolerated than gastric absorption [94]. Enteric coating of cysteamine allows for delayed absorption [95] and studies are underway to determine if it is possible to treat as effectively with a twice-a-day, rather than every 6-hour schedule [96, 97].

Treatment with carnitine rapidly reverses the low plasma levels of free-carnitine; however, muscle levels of carnitine may require years of treatment to reach the normal range [98, 99]. Despite this, the muscle may still contain Oil Red-O staining lipid droplets. Doses employed have approximated 100 mg/kg per day.

REFERENCES

1. Abderhalden E. Familiäre Cystindiähese, *Z Physiol Chem* 1903; **38**: 557.
2. Lignac GOE. Über Störung des Cystinstoffwechsels bei Kindern. *Deutsch Arch Klin Med* 1924; **145**: 139.
3. Fanconi G. Die nicht diabetischen Glykosurien und Hyperglykamien des altern Kindes. *Jahrb Kinderheilkd* 1931; **133**: 257.
4. de Toni G. Remarks on the relations between renal rickets (renal dwarfism) and renal diabetes. *Acta Paediatr* 1933; **16**: 479.
5. Debré R, Marie J, Cletet F, Messimy R. Rachitisme tardif coexistent avec une nephrite chronique et une glycosurie. *Arch Med Enf* 1934; **37**: 597.
6. Fanconi G. Der nephrotisch-glykosurische. Zwergwuchs mit hypophosphatamischer Rachitis. *Dtsch Med Wochenschr* 1936; **62**: 1169.
7. Dent CE. The amino-aciduria in Fanconi syndrome; a study making extensive use of techniques based on paper partition chromatography. *Biochem J* 1947; **41**: 240.
8. Fanconi G, Bickel H. Die chronische aminoacidurie (aminosäurediabetes oder nephrotisch-glukosurischer zergwuchs) bei der glykogenose und der cystinkrankheit. *Helv Paediatr Acta* 1949; **4**: 359.
9. Bickel H, Baar HS, Astley R. Cystine storage disease with aminoaciduria and dwarfism (Lignac-Fanconi Disease). *Acta Paed Uppsala* 1952; **42**: 1.
10. Schneider JA, Bradley K, Seegmiller JE. Increased cystine in leukocytes from individuals homozygous and heterozygous for cystinosis. *Science* 1967; **157**: 1321.
11. Schneider JA, Rosenbloom FM, Bradley KH, Seegmiller JE. Increased free-cystine content of fibroblasts cultured from patients with cystinosis. *Biochem Biophys Res Commun* 1967; **29**: 527.
12. Patrick AD, Lake BD. Cystinosis: electron microscopic evidence of lysosomal storage of cystine in lymph node. *J Clin Pathol* 1968; **21**: 571.
13. Harms E, Schneider JA. The lysosomal localization of free-cystine in normal cystinotic cells. *Clin Res* 1979; **27**: 457A.
14. Steinherz R, Tietze F, Gahl WA *et al.* Cystine accumulation and clearance by normal and cystinotic leukocytes exposed to cystine dimethyl ester. *Proc Natl Acad Sci USA* 1982; **79**: 4446.
15. Jonas AJ, Greene AA, Smith ML, Schneider JA. Cystine accumulation and loss in normal, heterozygous, and cystinotic fibroblasts. *Proc Natl Acad Sci USA* 1982; **79**: 4442.
16. Gahl WA, Bashan N, Tietze F *et al.* Cystine transport is defective in isolated leukocyte lysosomes from patients with cystinosis. *Science* 1982; **217**: 1263.
17. Jonas AJ, Smith ML, Schneider JA. ATP-dependent lysosomal cystine efflux is defective in cystinosis. *J Biol Chem* 1982; **257**: 13185.
18. Gahl WA, Thoene JG, Schneider JA. Cystinosis. *N Engl J Med* 2002; **347**: 111.
19. Seegmiller JE, Friedmann T, Harrison HE *et al.* Cystinosis. Combined clinical staff conference at the National Institutes of Health. *Ann Intern Med* 1968; **68**: 883.
20. Schulman JD. Cystinosis. DHEW Publication No (NIH) 72-249. Washington DC: US Government Printing Office, 1973.
21. Gahl WA. Cystinosis coming of age. *Adv Pediatr* 1986; **33**: 95.
22. Lebel M, Grose JH, Delage E, Crepin G. Syndrome de Bartter associé à une cystinose. Association des Médecins de la Langue Française du Canada, Congrès annuel, 1977: 5–8.
23. Lemire J, Kaplan BS, Scriver CR. Presentation of cystinosis as Bartter's syndrome and conversion to Fanconi syndrome on indomethacin treatment. *Pediatr Res* 1978; **12**: 544.
24. O'Regan S, Mongeau JG, Robitaille P. A patient with cystinosis presenting with the features of Bartter syndrome. *Acta Paediatr Belg* 1980; **33**: 51.
25. Waldmann TA, Mogielnicki RP, Strober W. The proteinuria of cystinosis: its pattern and pathogenesis. In: Schulman JD (ed.). *Cystinosis*. Washington DC: DHEW Publication, 1972.
26. Bernardini I, Rizzo WB, Dalakas M *et al.* Plasma and muscle free carnitine deficiency due to renal Fanconi syndrome. *J Clin Invest* 1985; **75**: 1124.

27. Black J, Stapleton FB, Roy S 3rd *et al.* Varied types of urinary calculi in a patient with cystinosis without renal tubular acidosis. *Pediatrics* 1986; **78**: 295.
28. Saleem MA, Milford DV, Alton H *et al.* Hypercalciuria and ultrasound abnormalities in children with cystinosis. *Pediatr Nephrol* 1995; **9**: 45.
29. Theodoropoulos DS, Shawker TH, Heinrichs C, Gahl WA. Medullary nephrocalcinosis in nephropathic cystinosis. *Pediatr Nephrol* 1995; **9**: 412.
30. Gretz N, Manz F, Augustin R *et al.* Survival time in cystinosis. A collaborative study. *Proc Eur Dial Transplant Assoc* 1983; **19**: 582.
31. Greco M, Brugnara M, Zaffanello M *et al.* Long-term outcome of nephropathic cystinosis: a 20-year single-center experience. *Pediatr Nephrol* 2010; **25**: 2459.
32. Gahl WA, Reed GF, Thoene JG *et al.* Cysteamine therapy for children with nephropathic cystinosis. *N Engl J Med* 1987; **316**: 971.
33. Gahl WA, Kaiser-Kupfer MI. Complications of nephropathic cystinosis after renal failure. *Pediatr Nephrol* 1987; **1**: 260.
34. Jonas AJ, Schneider JA. Cystinosis in a black child. *J Pediatr* 1982; **100**: 934.
35. Sochett E, Pettifor JM, Milner L *et al.* Nephropathic cystinosis in black children. Case reports. *SAfr Med J* 1984; **65**: 397.
36. Gahl WA, Hubbard VS, Orloff S. Decreased sweat production in cystinosis. *J Pediatr* 1984; **104**: 904.
37. Bürki E. Ueber die Cystinkrankheit im Kleinkindesalter unter besonderer Berücksichtigung des Augenbefundes. *Ophthalmologica* 1941; **101**: 257.
38. Schneider JA, Wong V, Seegmiller JE. The early diagnosis of cystinosis. *J Pediatr* 1969; **74**: 114.
39. Katz B, Melles RB, Schneider JA, Rao NA. Corneal thickness in nephropathic cystinosis. *Br J Ophthalmol* 1989; **73**: 665.
40. Korn D. Demonstration of cystine crystals in peripheral white blood cells in a patient with cystinosis. *N Engl J Med* 1960; **262**: 545.
41. Kaiser-Kupfer MI, Datiles MB, Gahl WA. Corneal transplant in boy with nephropathic cystinosis. *Lancet* 1987; **1**: 331.
42. Tsilou ET, Rubin BI, Reed GF *et al.* Age-related prevalence of anterior segment complications in patients with infantile nephropathic cystinosis. *Cornea* 2002; **21**: 173.
43. Frazier PD, Wong VG. Cystinosis. Histologic and crystallographic examination of crystals in eye tissues. *Arch Ophthalmol* 1968; **80**: 87.
44. Labbe A, Niaudet P, Loirat C *et al.* *In vivo* confocal microscopy and anterior segment optical coherence tomography analysis of the cornea in nephropathic cystinosis. *Ophthalmology* 2009; **116**: 870.
45. Wong VG, Lietman PS, Seegmiller JE. Alterations of pigment epithelium in cystinosis. *Arch Ophthalmol* 1967; **77**: 361.
46. Chan AM, Lynch MJ, Bailey JD *et al.* Hypothyroidism in cystinosis. A clinical, endocrinologic and histologic study involving sixteen patients with cystinosis. *Am J Med* 1970; **48**: 678.
47. Burke JR, El-Bishti MM, Maisey MN, Chantler C. Hypothyroidism in children with cystinosis. *Arch Dis Child* 1978; **53**: 947.
48. Czernichow P, Lenoir G, Roy MP *et al.* Thyroid involvement in cystinosis. *Arch Fr Pediatr* 1978; **35**: 930.
49. Gahl WA, Schneider JA, Thoene JG, Chesney R. Course of nephropathic cystinosis after age 10 years. *J Pediatr* 1986; **109**: 605.
50. Lucky AW, Howley PM, Megyesi K *et al.* Endocrine studies in cystinosis: compensated primary hypothyroidism. *J Pediatr* 1977; **91**: 204.
51. Fivush B, Green OC, Porter CC *et al.* Pancreatic endocrine insufficiency in posttransplant cystinosis. *Am J Dis Child* 1987; **141**: 1087.
52. Fivush B, Flick JA, Gahl WA. Pancreatic exocrine insufficiency in a patient with nephropathic cystinosis. *J Pediatr* 1988; **112**: 49.
53. Chik CL, Friedman A, Merriam GR, Gahl WA. Pituitary-testicular function in nephropathic cystinosis. *Ann Intern Med* 1993; **119**: 568.
54. Besouw MT, Kremer JA, Janssen MC, Levtschenko EN. Fertility status in male cystinosis patients treated with cysteamine. *Fertil Steril* 2010; **93**: 1880.
55. Reiss RE, Kuwabara T, Smith ML, Gahl WA. Successful pregnancy despite placental cystine crystals in a woman with nephropathic cystinosis. *N Engl J Med* 1988; **319**: 223.
56. Andrews PA, Sacks SH, van't Hoff W. Successful pregnancy in cystinosis. *J Am Med Assoc* 1994; **272**: 1327.
57. Charnas LR, Luciano CA, Dalakas M *et al.* Distal vacuolar myopathy in nephropathic cystinosis. *Ann Neurol* 1994; **35**: 181.
58. Ballantyne AO, Trauner DA. Neurobehavioral consequences of a genetic metabolic disorder: visual processing deficits in infantile nephropathic cystinosis. *Neuropsychiatry Neuropsychol Behav Neurol* 2000; **13**: 254.
59. Ehrich JH, Stoeppeler L, Offner G, Brodehl J. Evidence for cerebral involvement in nephropathic cystinosis. *Neuropadiatrie* 1979; **10**: 128.
60. Ehrich JH, Wolff G, Stoeppeler L *et al.* Psychosocial intellectual development of children with infantile cystinosis and cerebral atrophy (author's transl). *Klin Padiatr* 1979; **191**: 483.
61. Trauner DA, Williams J, Ballantyne AO *et al.* Neurological impairment in nephropathic cystinosis: motor coordination deficits. *Pediatr Nephrol* 2010; **25**: 2061.
62. Cochat P, Drachman R, Gagnadoux MF *et al.* Cerebral atrophy and nephropathic cystinosis. *Arch Dis Child* 1986; **61**: 401.
63. Fink JK, Brouwers P, Barton N *et al.* Neurologic complications in long-standing nephropathic cystinosis. *Arch Neurol* 1989; **46**: 543.
64. Lietman PS, Frazier PD, Wong VG *et al.* Adult cystinosis – a benign disorder. *Am J Med* 1966; **40**: 511.
65. Brubaker RF, Wong VG, Schulman JD *et al.* Benign cystinosis. The clinical, biochemical and morphologic findings in a family with two affected siblings. *Am J Med* 1970; **49**: 546.
66. Dodd MJ, Pusin SM, Green WR. Adult cystinosis. A case report. *Arch Ophthalmol* 1978; **96**: 1054.
67. Goldman H, Scriver CR, Aaron K *et al.* Adolescent cystinosis: comparisons with infantile and adult forms. *Pediatrics* 1971; **47**: 979.

68. Aaron K, Goldman H, Sriver CR. Cystinosis: new observations: 1. Adolescent (type III) form 2, correction of phenotypes *in vitro* with dithiothreitol. In: Carson NAJ, Raine DN (eds). *Inherited Disorders of Sulphur Metabolism*. Edinburgh: Churchill Livingstone, 1971: 150.
69. Spear GS, Slusser RJ, Schulman JD, Alexander F. Polykaryocytosis of the visceral glomerular epithelium in cystinosis with description of an unusual clinical variant. *Johns Hopkins Med J* 1971; **129**: 83.
70. Bois E, Feingold J, Frenay P, Briard ML. Infantile cystinosis in France: genetics, incidence, geographic distribution. *J Med Genet* 1976; **13**: 434.
71. Schneider JA, Wong V, Bradley K, Seegmiller JE. Biochemical comparisons of the adult and childhood forms of cystinosis. *N Engl J Med* 1968; **279**: 1253.
72. Jonas AJ, Smith ML, Allison WS *et al*. Proton-translocating ATPase and lysosomal cystine transport. *J Biol Chem* 1983; **258**: 11727.
73. Steinherz R, Tietze F, Triche T *et al*. Heterozygote detection in cystinosis, using leukocytes exposed to cystine dimethyl ester. *N Engl J Med* 1982; **306**: 1468.
74. Schneider JA, Bradley KH, Seegmiller JE. Transport and intracellular fate of cysteine-35S in leukocytes from normal subjects and patients with cystinosis. *Pediatr Res* 1968; **2**: 441.
75. Schulman JD, Fujimoto WY, Bradley KH, Seegmiller JE. Identification of heterozygous genotype for cystinosis *in utero* by a new pulse-labeling technique: preliminary report. *J Pediatr* 1970; **77**: 468.
76. Gahl WA, Dorfmann A, Evans MI *et al*. Chorionic biopsy in the prenatal diagnosis of nephropathic cystinosis. In: Fraccaro M, Simmoni G, Brambati B (eds). *First Trimester Fetal Diagnosis*. Berlin: Springer-Verlag, 1985: 260–2.
77. The Cystinosis Collaborative Research Group. Linkage of the gene for cystinosis to markers on the short arm of chromosome 17. *Nat Genet* 1995; **10**: 246.
78. Town M, Jean G, Cherqui S *et al*. A novel gene encoding an integral membrane protein is mutated in nephropathic cystinosis. *Nat Genet* 1998; **18**: 319.
79. Forestier L, Jean G, Attard M *et al*. Molecular characterization of CTNS deletions in nephropathic cystinosis: development of a PCR-based detection assay. *Am J Hum Genet* 1999; **65**: 353.
80. Kalatzis V, Cherqui S, Antignac C, Gasnier B. Cystinosin, the protein defective in cystinosis, is a H(+)-driven lysosomal cystine transporter. *EMBO J* 2001; **20**: 5940.
81. Stenson PD, Mort M, Ball EV *et al*. The Human Gene Mutation Database: 2008 update. *Genome Med* 2009; **1**: 13.
82. Touchman JW, Anikster Y, Dietrich NL *et al*. The genomic region encompassing the nephropathic cystinosis gene (CTNS): complete sequencing of a 200-kb segment and discovery of a novel gene within the common cystinosis-causing deletion. *Genome Res* 2000; **10**: 165.
83. Anikster Y, Lucero C, Touchman JW *et al*. Identification and detection of the common 65-kb deletion breakpoint in the nephropathic cystinosis gene (CTNS). *Mol Genet Metab* 1999; **66**: 111.
84. Shotelersuk V, Larson D, Anikster Y *et al*. CTNS mutations in an American-based population of cystinosis patients. *Am J Hum Genet* 1998; **63**: 1352.
85. Phornphutkul C, Anikster Y, Huizing M *et al*. The promoter of a lysosomal membrane transporter gene, CTNS, binds Sp-1, shares sequences with the promoter of an adjacent gene, CARKL, and causes cystinosis if mutated in a critical region. *Am J Hum Genet* 2001; **69**: 712.
86. Theodoropoulos DS, Krasnewich D, Kaiser-Kupfer MI, Gahl WA. Classic nephropathic cystinosis as an adult disease. *J Am Med Assoc* 1993; **270**: 2200.
87. Thoene JG, Oshima RG, Crawhall JC *et al*. Cystinosis. Intracellular cystine depletion by aminothiols *in vitro* and *in vivo*. *J Clin Invest* 1976; **58**: 180–9.
88. Reznik VM, Adamson M, Adelman RD *et al*. Treatment of cystinosis with cysteamine from early infancy. *J Pediatr* 1991; **119**: 491.
89. Kleta R, Bernardini I, Ueda M *et al*. Long-term follow-up of well-treated nephropathic cystinosis patients. *J Pediatr* 2004; **145**: 555.
90. Kimonis VE, Troendle J, Rose SR *et al*. Effects of early cysteamine therapy on thyroid function and growth in nephropathic cystinosis. *J Clin Endocrinol Metab* 1995; **80**: 3257.
91. Dohil R, Newbury RO, Sellers ZM *et al*. The evaluation and treatment of gastrointestinal disease in children with cystinosis receiving cysteamine. *J Pediatr* 2003; **143**: 224.
92. Thoene JG, Lemons R. Cystine depletion of cystinotic tissues by phosphocysteamine (WR638). *J Pediatr* 1980; **96**: 1043.
93. Kaiser-Kupfer MI, Fujikawa L, Kuwabara T *et al*. Removal of corneal crystals by topical cysteamine in nephropathic cystinosis. *N Engl J Med* 1987; **316**: 775.
94. Dohil R, Fidler M, Barshop BA *et al*. Understanding intestinal cysteamine bitartrate absorption. *J Pediatr* 2006; **148**: 764.
95. Gangoi JA, Fidler M, Cabrera BL *et al*. Pharmacokinetics of enteric-coated cysteamine bitartrate in healthy adults: a pilot study. *Br J Clin Pharmacol* 2010; **70**: 376.
96. Dohil R, Fidler M, Gangoi JA *et al*. Twice-daily cysteamine bitartrate therapy for children with cystinosis. *J Pediatr* 2010; **156**: 71.
97. Dohil R, Gangoi JA, Cabrera BL *et al*. Long-term treatment of cystinosis in children with twice-daily cysteamine. *J Pediatr* 2010; **156**: 823.
98. Gahl WA, Bernardini I, Dalakas M *et al*. Oral carnitine therapy in children with cystinosis and renal Fanconi syndrome. *J Clin Invest* 1988; **81**: 549.
99. Gahl WA, Bernardini IM, Dalakas MC *et al*. Muscle carnitine repletion by long-term carnitine supplementation in nephropathic cystinosis. *Pediatr Res* 1993; **34**: 115.

Hartnup disease

Introduction	540	Treatment	542
Clinical abnormalities	540	References	542
Genetics and pathogenesis	542		

MAJOR PHENOTYPIC EXPRESSION

Cerebellar ataxia, pellagra-like photosensitive dermatosis, headaches, impaired mental development, psychiatric abnormalities; a specific generalized amino aciduria in which small, neutral monoamino monocarboxylic amino acids predominate, but glycine is normal and imino acids are absent; malabsorption of tryptophan which leads to indicanuria; and mutations in the SL6A19 transporter.

INTRODUCTION

Hartnup disease is a genetically determined disorder in which the renal tubular and intestinal transport of tryptophan and other ring-containing and neutral amino acids is impaired. It was named for the surname of the family in which the disorder was discovered [1, 2].

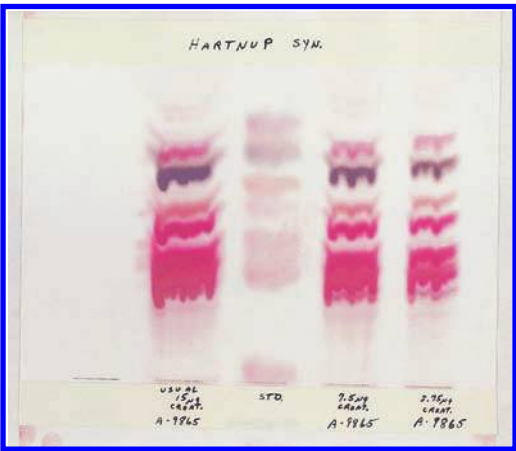


Figure 72.1 High voltage electropherogram of the amino acids of the urine of a patient with Hartnup disease. The gross aminoaciduria is evident in the first column, in which the usual sample containing 15 mg creatinine was run. Column 2 were standards. The other two runs were with 7.5 and 3.75 mg of creatinine, respectively. The dark band at the top is histidine, the next glycine, followed by alanine, glutamine, and serine.

Many individuals with Hartnup disease are asymptomatic. The single constant phenotypic feature is the unusual amino aciduria in which a large group of neutral α -amino acids are excreted in large quantities. The pattern was first recognized in paper partition chromatograms [1–3], but it is also clearly recognizable on electrophoresis (Figure 72.1) and on quantitative analysis of the amino acids of the urine in the amino acid analyzer (Figure 72.2). Deficient activity of a sodium-dependent transporter, which controls the absorption of these amino acids at the brush-border of the intestinal and renal epithelium, leads to the phenotype.

The gene was mapped in the original family by Kleta and colleagues [4] to chromosome 5p15. They sequenced the candidate gene SLC6A19 in six families and identified five mutations. Seow *et al.* [5] also identified the gene and reported six mutations. They refined mapping to 5p15.33. The two papers were adjacent in *Nature Genetics* in 2004. Most patients were compound heterozygotes.

CLINICAL ABNORMALITIES

The clinical manifestations of the disease appear to be the result of a deficiency of tryptophan that is a consequence of the defect in its absorption. Failure to thrive could be a reflection of failure to absorb other amino acids as well. A patient of ours failed to thrive despite what was described as a good appetite until the diagnosis was made and treatment initiated.

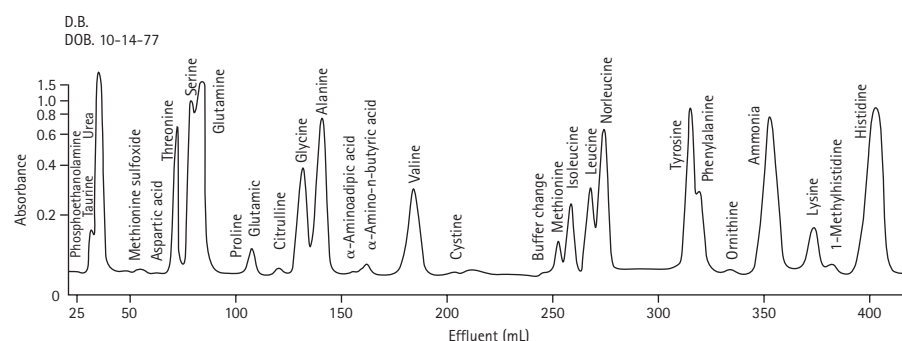


Figure 72.2 Amino acid analyzer chromatogram of the urine of the same patient. The characteristic pattern is of the massive excretion of neutral monoaminomonocarboxylic acids including alanine, serine, threonine, and histidine.

The most dramatic expression of the disease is with a red, scaly, pellagra-like dermatosis and cerebellar ataxia [1, 2]. The rash has a predilection for exposed areas of the body and is clearly photosensitive. It can be extensive in patients with considerable exposure to ultraviolet light. Two patients [2] had hydroa vacciniforme, or vesiculobullous and erythematous photodermatitis with persistent scarring. The involved areas may remain depigmented. Some patients have had glossitis.

Ataxia may be seen in the absence of dermatosis, and it may be episodic. During attacks, the patient may be unsteady and walk with a wide-based gait. Our patient was developmentally slow, and his siblings were not; at 8 years he could readily be shown to be ataxic on finger to nose, heel to shin, or standing on one foot, and to have dysidiadochokinesia, despite good compliance with treatment. Intention tremor and nystagmus may be present. There may be diplopia. Some patients may have persistent headaches. The original patient had impaired mental development, but this was not regularly seen in other involved members of the family. Nine of 39 reported patients [2] had mildly impaired mental development. Others have been reported with impairment but no other symptoms [6]. In a study of 21 affected individuals identified through newborn screening, there were no differences in IQ or percentile levels of growth from those of 19 control siblings [7]. However, each of the two with poor academic performance scores encountered were in the Hartnup group, and two others in this group were considered to have learning difficulties. Two of these had clinical manifestations: in one it was described as a 'pellagrin' episode, in which an eczematoid eruption over the body and thighs associated with edema followed a prolonged episode of diarrhea; and in the other there was somewhat impaired growth and intellectual development – his weight was in the third percentile and IQ was 97, but his two siblings scored 120. On the other hand, the disease does not seem to cause progressive mental defect, and some involved patients have been highly intelligent. The electroencephalogram may be abnormal.

Psychiatric symptoms may be dramatic, as in pellagra. The disorder may be discovered in patients with intermittent psychiatric abnormalities. Symptoms have ranged from mild emotional lability to severe delirium. Patients have been reported with delusions and with vivid

hallucinations or depersonalization. A Japanese patient had psychiatric manifestations, ataxia, and diplopia which went away after treatment with niacin [8].

The disorder has been observed throughout the world, but until the advent of neonatal screening programs, such as the one in Massachusetts where the infant's urine was examined, it was rarely encountered in North America, where general levels of nutrition are high. This has led to the idea that marginal nutritional intake, especially of niacin or protein, may predispose an affected individual to the development of clinical manifestations. This issue and the greater requirements for growth could also explain the much more common occurrence of clinical manifestations in children than in adults. Certainly, exposure to sunlight is important in the development of dermatological features of the phenotype. Sulfonamides have also been thought to be precipitating agents.

The diagnosis has classically been made by examination of the pattern of excretion of amino acids in the urine. The aminoaciduria is generalized, but it differs from the usual nonspecific generalized aminoaciduria in that glycine and the imino acids, proline and hydroxyproline, are not excreted in unusual quantity. The amino acids excreted in greatest quantity are those, excluding glycine, that are found in largest quantity in normal urine – alanine, glutamine, serine, and histidine. Amino acids, many of them essential, which are excreted in very small quantities in normal urine but in prominent amounts in the urine of patients with Hartnup disease, include leucine, isoleucine, valine, threonine, phenylalanine, tyrosine, and tryptophan. The tryptophanuria was missed when the patient is studied only by column chromatography on early amino acid analyzers because tryptophan was destroyed by the conditions of analysis, but it can be quantified on modern analyzers. Asparagine is also excreted in increased quantities; this compound was not usually separated from glutamine by the original amino acid analyzers, but it is currently well separated, it is also shown in paper chromatograms. The concentrations of these amino acids in the blood are normal or somewhat reduced [9].

A variety of indolyl derivatives of tryptophan is also found in the urine (Figure 72.3). The most prominent is indican. Patients have been reported to excrete as much as 400 mg of indoxylsulfate a day. They may also excrete increased amounts of indoxylglucuronide. Indolylacetic

7. Scriver CR, Mahon B, Levy HL *et al*. The Hartnup phenotype: Mendelian transport disorder multifactorial disease. *Am J Hum Genet* 1987; **40**: 401.
8. Nozaki J, Dakeishi M, Ohura T *et al*. Homozygosity mapping to chromosome 5p15 of a gene responsible for Hartnup disorder. *Biochem Res Commun* 2001; **284**: 255.
9. Cusworth DC, Dent CE. Renal clearances of amino acids in normal adults and in patients with aminoaciduria. *Biochem J* 1960; **74**: 551.
10. Milne MD, Crawford MA, Girao CB, Loughridge LW. The metabolic disorder in Hartnup disease. *Q J Med* 1960; **29**: 407.
11. Hillman RE, Steward A, Miles JH. Amino acid transport defect in intestine not affecting kidney. *Pediatr Res* 1986; **20**: 265A.
12. Jonxis JHP. Oligophrenia phenylpyruvica en de hartnupziekte. *Ned Tijdschr Geneesk* 1957; **101**: 569.
13. Tada K, Hirono H, Arakawa T. Endogenous renal clearance rates of free amino acids in prolinuric and Hartnup patients. *Tohoku J Exp Med* 1967; **93**: 57.
14. Halvorsen S, Hygstedt O, Jagenburg R, Sjaastad O. Cellular transport of L-histidine in Hartnup disease. *J Clin Invest* 1969; **48**: 552.
15. Christensen HN. Organic ion transport during seven decades. The amino acids. *Biochim Biophys Acta* 1984; **779**: 255.
16. Broer A, Klingel K, Kowalczyk S *et al*. Molecular cloning of mouse amino acid transporter system B(0), a neutral amino acid transporter related to Hartnup disorder. *J Biol Chem* 2004; **279**: 24 467.
17. Gleason WA, Butler JJ, Jonas AJ. Long term therapy of Hartnup disorder with tryptophan ethylester. *Pediatr Res* 1992; **31**: 348A.

Histidinuria

Introduction	544	Treatment	545
Clinical abnormalities	544	References	545
Genetics and pathogenesis	545		

MAJOR PHENOTYPIC EXPRESSION

Increased excretion of histidine in urine without histidinemia and impaired intestinal absorption of histidine.

INTRODUCTION

Isolated histidinuria is a rare disorder of histidine transport. It has been reported in six individuals in five families [1–6]. There may be no other abnormalities, but four of the patients reported had some abnormality of the central nervous system.

CLINICAL ABNORMALITIES

Our patient [1] had a distinctive phenotype. He was moderately developmentally delayed and had bilateral neural hearing loss. He had a history of substantial neonatal hypoglycemia. In addition, he had a number of minor anomalies (Figures 73.1 and 73.2). The shortness of the fifth fingers resulted from a short, rounded middle phalanx.



Figure 73.1 DB: An eight-year-old boy with histidinuria. External ears were large, protuberant, and somewhat simple. The nasal bridge was broad and the philtrum long and smooth, and the upper lip was thin. He had bilateral nerve deafness. (This illustration is reprinted with permission from the *American Journal of Medical Genetics* [1].)



Figure 73.2 The hands of DB illustrate the very small fifth fingers, which had only two horizontal creases, despite three phalanges on each hand. The first metacarpals were short. (This illustration is reprinted with permission from the *American Journal of Medical Genetics* [1].)

The other patients reported had a variety of abnormalities of the central nervous system. Two siblings had mildly impaired mental development [3]. Another was severely mentally impaired and had microcephaly and spastic diplegia [4]. The fourth was intellectually normal, but he developed myoclonic seizures at the age of 13 years [2]. It appears unlikely that any of these abnormalities are consequences of abnormal transport of histidine; most probably they represent bias of ascertainment.

GENETICS AND PATHOGENESIS

Recessive inheritance seems likely, as the parents of all patients were normal, and there were siblings. The four patients were all male, and so X-linked inheritance is a possibility, but an autosomal gene is not ruled out. The consistent occurrence of abnormality of the central nervous system could represent the possibility of deletion of adjacent closely linked genes, or the pleiotropic effects of a single gene.

The hallmark of this disease is an elevated level of excretion of histidine in the urine. In our patient, this was 276 mmol/mol creatinine. This was approximately twice the upper limit of normal for our laboratory. In another patient [4], the level was 1.5 times the upper limit of normal and 2.5 times the control mean. In the others, data were not reported in terms of creatinine excretion, but total 24-hour excretion was two to three times the control

mean. In patients with histidinemia, urinary excretion was seven times the control mean.

In contrast to patients with histidinemia, the patients tend to have low concentrations of histidine in plasma. In the patients reported [1–5], plasma concentrations ranged from 64 to 87 $\mu\text{mol/L}$. In our laboratory, the normal range is 50–100 $\mu\text{mol/L}$, and the reported mean of 36 control subjects aged three to ten years was 72 ± 19 [7]. Histidine concentration in the cerebrospinal fluid was normal in the patient in whom it was measured [4]. One patient, a developmentally normal female, presented with cataracts at 17 years of age [6].

Intestinal absorption is, in our view, also low in these individuals. Figure 73.3 illustrates the response to oral loading in a patient. In these patients, peak levels were not only lower; they were also achieved later than in controls.

TREATMENT

Treatment is not indicated for this abnormality in transport. Dietary sources of histidine are adequate to compensate and prevent symptoms of deficiency.

REFERENCES

1. Nyhan WL, Hilton S. Histidinuria: defective transport of histidine. *Am J Med Genet* 1992; **44**: 558.
2. Holmgren G, Hambraeus L, de Chateau P. Histidinemia and normo-histidinemic histinuria. *Acta Paediatr Scand* 1974; **63**: 220.
3. Sabater J, Ferre C, Pulio M, Maya A. Histidinuria: a renal and intestinal histidine transport deficiency found in two mentally retarded children. *Clin Genet* 1976; **9**: 117.
4. Kamoun PP, Parry P, Cathelineau L. Renal histidinuria. *J Inherit Metab Dis* 1981; **4**: 217.
5. Scriver CR, Tenenhouse HS. Mendelian phenotypes as 'probes' of renal transport systems for amino acids and phosphate. In: Windhager E (ed.). *Handbook of Physiology* Section 8: Renal Physiology. New York: Oxford University Press, 1992: 1977.
6. Santacana-Laffitte G, Izquierdo NJ, Lladó JR, Maumenee IH. Juvenile cataracts in a patient with histidinuria: case report. *Ophthalmic Genet* 2006; **27**: 161–3.
7. Ghisolfi J, Augier D, Regnier C, Dalous A. Etude des variations physiologiques en fonction de l'âge du taux des acides libres plasmatiques chez l'enfant normal. *Arch Franc Ped* 1973; **30**: 951.

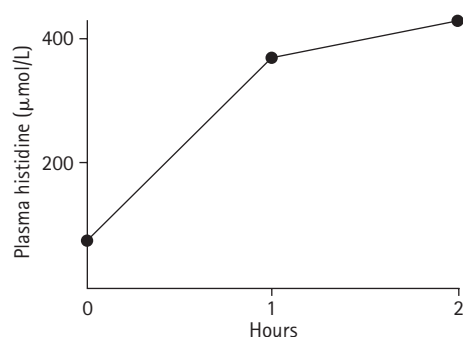


Figure 73.3 Plasma concentrations of histidine in response to 100 mg/kg of oral histidine indicate impaired intestinal transport in the patient. (This illustration is reprinted with permission from the *American Journal of Medical Genetics* [1].)

Menkes disease

Introduction	546	Treatment	550
Clinical abnormalities	547	References	550
Genetics and pathogenesis	549		

MAJOR PHENOTYPIC EXPRESSION

Progressive early-onset cerebral degeneration; convulsions; depigmented, steel wool-like hair; bone lesions like those of scurvy; elongated, tortuous cerebral arteries; hypocupremia; diminished intestinal absorption of copper; increased concentrations of copper in intestinal mucosal cells and cultured fibroblasts; and defective activity of the copper-transporting adenosine triphosphatase (ATPase) (ATP7A).

INTRODUCTION

In 1962, Menkes and colleagues [1] described a disorder in five patients in which progressive cerebral deterioration was associated with characteristic abnormalities of the hair. The hair has been referred to as kinky [2], but it is anything but kinky. It is short and tends to stand on end, assuming a brush-like appearance (Figures 74.1 and 74.2). It has been called steely hair by Danks [3]. It looks to us something like steel wool, but in Australia the wool of copper-deficient sheep has long been called steely. It was recognized in the initial report [1] that the disease was transmitted as an X-linked recessive trait. It was the recognition by Danks and colleagues [4, 5] that the hair resembled the appearance of wool of copper-deficient sheep that led to their characterization of the disease as a disorder of copper transport. Copper deficiency in sheep leads to deficiency in the formation of cross-linking disulfide bonds in the keratin of the wool.

Abnormal transport of copper can be demonstrated in cultured fibroblasts which accumulate labeled copper, illustrating the failure of efflux also noted in intestinal cells [6, 7]. The gene has been isolated from the Xq13 locus by three independent groups and shown to code for a copper-transporting P-type ATPase with six copper binding sites at the amino terminal end [8–10]. The ATPase, ATP7A is located on the trans-Golgi membrane of all human cells except hepatocyte. Thus in Menkes disease, transport of copper from cytosol to Golgi is deficient, and enzymes that

require copper and incorporate this copper in the Golgi are defective in activity because of the copper deficiency in the organelle [11–17]. Deletions in the gene were found early in patients with the disease [9], but a complete spectrum



Figure 74.1 JE: An infant with Menkes disease. The hair was striking. (Illustrations of this patient were kindly provided by Dr Marilyn Jones of UCSD and Children's Hospital and HealthCenter, San Diego.)



Figure 74.2 JE: In this view, the unusual hair is seen even more clearly.

of mutation has been observed, most of them unique to an individual family [12–15].

CLINICAL ABNORMALITIES

The most important feature of the disease is its effect on the central nervous system. These patients usually appear normal at birth, although a number of them are born prematurely. There may be neonatal hypothermia and hyperbilirubinemia. The hair may be fine, but neonatal problems may resolve. The infants may grow and develop normally for 6–12 weeks, but vomiting, difficulty with feeding and poor weight gain are frequently encountered early. The onset of cerebral manifestations may be ushered in by seizures, or the patient may simply be noted to be apathetic, lethargic, or hypotonic and to display little interest in feedings. Rapid progressive neurologic deterioration ensues, with loss of milestones achieved. The ultimate state is flaccid hypotonia. There are few spontaneous movements and no contact with the environment. Seizures are generalized and frequent; and may be refractory to treatment. Myoclonic seizures may be seen as well as major motor seizures. Spastic quadriparesis may develop, and there may be opisthotonos and scissoring. Loss of visual ability and of hearing have been described.

The hair is a striking diagnostic feature (Figures 74.1, 74.2, 74.3, 74.4, and 74.5 and Table 74.1). It tends to be

Table 74.1 Abnormalities of the hair

Menkes disease, pili torti, trichorrhexis nodosa, monilethrix
Kinky hair, photosensitivity and impaired mental development
Argininosuccinic aciduria
Pili torti – isolated, with deafness or with dental enamel hypoplasia, MIM 261900
Trichothiodystrophy – trichorrhexis nodosa, ichthyosis and neurologic abnormalities (Pollit syndrome) MIM 27550



Figure 74.3 NS: An infant with Menkes disease. The hair had been cut; what was left was straight and wiry.



Figure 74.4 RE: A boy with Menkes disease. The hair was short and brittle and under the microscope displayed pili torti. He was globally delayed and had spasticity. The legs assumed a scissor position.

short because hairs break off readily, especially at places of contact with the bed. It may be tangled and unkempt, looking stringy, or brush-like. It may be poorly pigmented, white or lackluster. Under the microscope the appearance may be that of pili torti (Figure 74.5) in which the hair shafts are twisted. They may also have the brush-like microscopic appearance of trichorrhexis nodosa or the segmental narrowing of monilethrix.

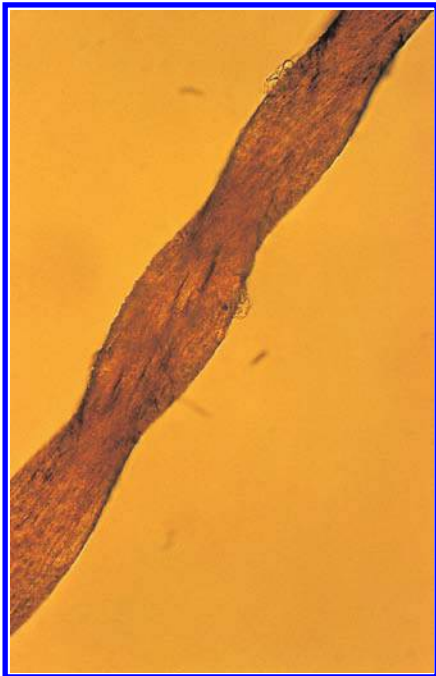


Figure 74.5 Pili torti in the patient shown in Figure 74.3.

The skin may appear pale or pasty, thick, or pudgy, especially in the cheeks. The jowels may sag, and there may be other signs of cutis laxa. The nasal bridge may be broad and there may be epicanthal folds. Hair of the eyebrows is also abnormal. Somatic growth is usually impaired.

The roentgenographic appearance of the bones is also characteristic. In addition to general osteoporosis in an irregular lucent trabecular pattern, there are spurs over flared metaphyseal areas that fracture and produce a scorbutic appearance [5, 18]. Flaring of the ribs is like that seen in rickets and the ribs commonly fracture. Diaphyseal periosteal reaction along with fractures [19] may suggest a diagnosis of nonaccidental trauma [18]. This impression may be strengthened by finding subdural hematomas, which also occur in this disease [20]. Multiple wormian bones in the cranial sutures complete the characteristic roentgenographic picture [21].

Abnormalities of the electroencephalogram (EEG) are regularly seen. Multifocal spike patterns may eventually be replaced by a pattern of hypsarrhythmia. Visual and auditory evoked responses may disappear. Serial computed tomography (CT) scans in four patients [20] revealed a considerable variability in spite of similar general clinical courses. Early in the disease the scan may appear normal. Progressive abnormalities, especially in the white matter, have also been demonstrated by magnetic resonance imaging (MRI) [22]. Angiography reveals elongated and tortuous intracranial and visceral vessels. Multiple areas of localized narrowing are seen, and these abnormal changes in cerebral vessels may also be visualized by MRI. Diffuse cortical atrophy may be seen, or there may be multifocal areas of destruction of brain as a result of ischemic infarction (Table 74.2). In some patients, the rate of growth of the head falls off dramatically, resulting in microcephaly. Autopsy may reveal general cerebral and cerebellar atrophy, or loss of brain substance may be strikingly focal with evidence of infarction and later gliosis or cyst formation. These changes are consistent with the vascular abnormalities that characterize the disease [5, 23, 24]. The basic lesion appears to be a defect in the elastic fibers of the vessels; localized and similar changes are seen in the skin [23]. Much of the cerebral pathology may be secondary to the vascular abnormalities. Similar changes are seen in vessels in the viscera and extremities. Vessels may have localized areas of narrowing and dilatation. Vascular occlusion may occur anywhere. In one of our patients, thrombosis occluded both jugular veins in the neonatal period.

Connective tissue abnormality may also be evident in diverticulae of the bladder or ureters, and these may rupture or lead to urinary tract infections. Cystography may show a large, trabeculated bladder [25]. There may be umbilical hernia. Major gastrointestinal bleeding has been reported [26], arising from gastric polyps associated with underlying vascular ectasis and mucosal redundancy.

Most patients die between three and 12 months. Some survive until three years. A rare patient may survive as long as 12 years of age [25]. Two patients have been reported in whom there was a milder phenotype [17, 27–29].

Table 74.2 Metabolic disease and stroke

Stroke (conventional)	Metabolic stroke
Congenital disorders of glycosylation	Carbamyl phosphate synthase deficiency
Ethylmalonic aciduria	Glutaric acidemia type I
Fabry disease	Isovaleric acidemia
Homocystinuria	MELAS (Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes)
Menkes disease	α -Methylene tetrahydrofolate reductase deficiency
Purine nucleoside phosphorylase deficiency	Methylcrotonyl CoA carboxylase
	Methylmalonic acidemia deficiency
	Ornithine transcarbamylase deficiency

One presented at two years of age with ataxia and mild developmental delay [27]; changes in the bones were mild, but there was pili torti, and aortography indicated elongation and dilatation of the arteries. He was treated with parenteral copper, but it is not clear that it altered his disease [28]. By 14 years, he was severely ataxic and had progressed slowly in school for his age. The other patient presented with recurrent urinary tract infection and diverticulae of the tract [29]. He had pili torti and mild ataxia; the facial appearance was that of Menkes disease, with loose skin and sagging jowels, and the joints were lax. Arteriography revealed only mild dilatation of vessels.

Another variant in which copper transport in fibroblasts is indistinguishable from Menkes disease is occipital horn disease [30–38]. This disorder has been referred to as X-linked cutis laxa [30] and Ehlers-Danlos syndrome type IX [34]. It is characterized by cutis laxa, hernias, and diverticulae of the bladder or ureters. Some patients have had chronic diarrhea [34]. Roentgenographic findings include the characteristic ossified occipital horns, which are palpable, hammer-like extensions pointing caudally on the sides of the foramen magnum. In addition, the lateral ends of the clavicles are broad and the cortices of long bones are wavy [34]. Intellect may be a little low.

The histopathology of Menkes disease is impressive for changes in the elastin lamina of the vessels, which are fragmented, disrupted, or reduplicated [5]. The intima may be thickened. Electron microscopy suggests defective formation of elastin [39]. The cerebrum and cerebellum are notable for widespread neuronal loss and gliosis [1, 39, 40]. In muscle, iris, and retina [41–43], there is mitochondrial disorganization, and ragged red fibers have been reported [42, 43].

GENETICS AND PATHOGENESIS

Menkes disease and each of the variants are transmitted as X-linked recessive traits. Prevalence figures for Menkes disease have ranged from one in 35,000 in Melbourne [5] to one in 250,000 in Europe [16, 43].

Mutant strains of mice have been studied that appear to be excellent models [44–49]. The brindled and blotchy mice (Mobr and Mobl) are determined by allelic mutations in genes on the X-chromosome. The genes in the mouse map close to the tabby locus and nearer to α -galactosidase and phosphoglycerate kinase [47, 48]. Another (dappled) mutant is a prenatal lethal (Modp). The macular mouse with the mutant mottled gene has been shown to have a C to T4223 change leading to serine 1382 proline in the transmembrane domain of the copper transporter [49]. Tissue distribution of expression was consistent with the pathology of Menkes disease.

The human gene is located at Xq13. It has been found to be large, spanning approximately 140 kb over 23 exons [8–10, 50, 51]. The coding sequence predicted a structure homologous with that of the P-type cation-

transporting family of ATPase enzyme [52]. In addition to copper binding motifs, it contains a dicysteine membrane spanning area. The mouse gene is expressed in most tissues. No mRNA was found in the Modp, a normal amount of normal message in Mobr and mottled, and structurally abnormal mRNA in Mobl. In patients with Menkes disease, greatly reduced or undetectable levels of mRNA have been frequently observed [8–10]. Deletions or rearrangements in the gene have been observed in almost half of these patients [9, 13], and 1–2 percent have cytogenetically demonstrable abnormalities, such as translocations. Reduced levels of mRNA have also been observed in occipital horn syndrome [53], but Northern analysis of mRNA in most mild patients appeared normal [54]. Splicing mutations in milder patients suggest that some normal protein is made. The gene codes for an 8 kb transcript that is expressed in all cells but those of the liver. The amino acid sequence of the Menkes protein is 55 percent identical to the Wilson protein and contains the same functional motifs. The synthesized polypeptide is N-glycosylated and localized to the trans-Golgi network.

The abnormal gene causes abnormal transport of copper. Altered handling of copper has been documented in cultured fibroblasts [6, 7, 55–60] in which copper content is abnormally high, accumulation of ^{64}Cu is elevated and efflux of ^{64}Cu to isotope-free medium is impaired.

Heterozygotes may be recognized by the abnormality in copper processing in cultured fibroblasts, but the result may be normal [6, 55, 57, 61]. Cloning has revealed the presence of two cell populations [62]. Pili torti may also be recognized in some heterozygotes [63], and mosaic pigmentation of the skin has been observed in a black heterozygote [64].

The complete clinical expression of disease has been reported in a number of girls, in most of whom cytogenetic studies were normal [65–67]; one was the sister of an affected male [65]. In one, the patient was a mosaic 45X/46XX [67]. Another girl had a balanced 2/X translocation in which the break point was at Xq13.2-13.3 and proximal to PGK-1 [68, 69].

Prenatal monitoring has been carried out by the study of copper transport in cultured amniocytes or chorionic villus samples [6, 70]. The method is tricky and very sensitive to conditions of culture and assay.

The immediate consequence of the basic defect is impaired absorption of copper because of failure of transfer into the secretory pathway causing accumulation of copper in cells of the intestine and of the blood-brain barrier and generalized deficiency of copper in the blood and central nervous system. Measured concentrations of copper in the blood are very low, and so are those of ceruloplasmin [4, 5]. In normal individuals the serum contains over 12 μmol copper/L, while in most patients the levels were less than 6 μmol /L. Normal ceruloplasmin is over 20 mg/dL, while patients have less than 5 mg/dL. Over 80 percent of the copper in human plasma is in the form of ceruloplasmin. The copper contents of the liver and the brain are also

low. Oral administration of copper indicates an absorptive abnormality in these patients. The content of copper in the intestinal mucosa is high [3,71], a clear index that the defect is in transport out of the intestinal cell. The generality of the transport defect is indicated not only by the abnormality in cultured fibroblasts, but also in the kidney, spleen, lung, muscle, and pancreas [57]. In the case of an affected fetus, the placenta can also be shown to have a high content of copper, permitting confirmation of the prenatal diagnosis [72]. When the mutation is known, molecular analysis is the procedure of choice for prenatal diagnosis and heterozygote detection [73,74]. Red blood cell copper content is normal; there is no anemia or neutropenia, and the erythrocyte activity of superoxide dismutase is not reduced.

A shortage of copper in tissues leads to defective cross-linking of elastin and collagen, because lysyl oxidase is a copper-dependent enzyme, and lysyl oxidase activity is deficient in this syndrome [31, 75–77]. The deficiency of this enzyme is consistent with the abnormalities in connective tissue in Menkes disease. It is because of the defect in lysyl oxidase that this disorder has been classified as Ehlers-Danlos syndrome type IX. Other copper-dependent enzymes are also deficient in activity. Deficiency of tyrosinase leads to the lack of pigmentation in these patients [77]. The abnormal hair results from defective bonding of disulfides in keratin [4, 5]. Cytochrome oxidase deficiency has been demonstrated in mitochondria of patients with Menkes disease [78]. Elevated concentrations of lactic acid have been found in the cerebrospinal fluid. In a study of metabolites of collagen [19], urinary deoxypyridinoline was very low, and this has been proposed as a marker for the lysyloxidase deficiency in this disease.

TREATMENT

Treatment for this disease has been less than satisfactory. It is possible to administer sufficient copper intravenously or subcutaneously to bring concentrations of copper and ceruloplasmin in plasma and even liver to normal, and chronic therapy has been employed, but there has been no evident clinical influence on the course of the cerebral disease [79]. Concentrations of copper in the brain have not been brought to the normal range. Some success has been reported with copper-histidinate started soon after birth and continued for six and 15 years [80]; intellectual levels were at the lower end of the normal range, and there were abnormalities of hair and connective tissue. In a series of patients treated with subcutaneous copper histidinate, two patients treated from the first month for six and 16 years were judged to have done well neurologically [81]. Actually, the 16-year-old patient had an IQ of 87, but required a wheelchair because of extreme hypotonia and hyperextensibility of the joints. Five patients treated from the second to seventh month did poorly. Histidine enhances the uptake of copper by cells from plasma. It is generally considered that the therapy should be offered

to infants diagnosed early. Copper histidinate therapy has been initiated *in utero* [82]. It is clear that many effects of the disease begin *in utero*. Follow-up information is limited.

The mineral density of bone was reported to be improved in three patients treated with pamidronate intravenously for a year [83].

REFERENCES

1. Menkes JH, Alter M, Steigleder GK *et al*. A sex-linked recessive disorder with retardation of growth peculiar hair and focal cerebral and cerebellar degeneration. *Pediatrics* 1962; **29**: 764.
2. French JH, Sherard EA. Studies of the biochemical basis of kinky hair disease. *Pediatr Res* 1967; **1**: 206.
3. Danks DM, Cartwright E, Stevens BJ. Menkes' steely-hair (kinky-hair) disease. *Lancet* 1973; **1**: 891.
4. Danks DM, Campbell PE, Walker-Smith J *et al*. Menkes's kinky hair syndrome. *Lancet* 1972; **1**: 1100.
5. Danks DM, Campbell PE, Mayne V, Cartwright E. Menkes's kinky hair syndrome. An inherited defect in copper absorption with widespread effects. *Pediatrics* 1972; **50**: 188.
6. Horn N. Copper incorporation studies on cultured cells for prenatal diagnosis of Menkes' disease. *Lancet* 1976; **1**: 1156.
7. Chan WY, Garnica AD, Rennert OM. Cell culture studies of Menkes kinky hair disease. *Clin Chim Acta* 1978; **88**: 495.
8. Vulpe C, Levinson B, Whitney S *et al*. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. *Nat Genet* 1993; **3**: 7.
9. Chelly J, Tümer Z, Tonnesen T *et al*. Isolation of a candidate gene for Menkes disease that encodes a potential heavy metal binding protein. *Nat Genet* 1993; **3**: 14.
10. Mercer JFB, Livingston J, Hall B *et al*. Isolation of a partial candidate gene for Menkes disease by positional cloning. *Nat Genet* 1993; **3**: 20.
11. Kodama H, Murata Y. Molecular genetics and pathophysiology of Menkes' disease. *Pediatr Int* 1999; **41**: 430.
12. Zeynep T, Horn M. Menkes disease: recent advances and new aspects. *J Med Genet* 1997; **34**: 265.
13. Das S, Levinson B, Whitney S *et al*. Diverse mutations in patients with Menkes disease often lead to exon skipping. *Am J Hum Genet* 1994; **55**: 883.
14. Tümer Z, Lund C, Tolshave J *et al*. Identification of point mutations in 41 unrelated patients affected with Menkes disease. *Am J Hum Genet* 1997; **60**: 63.
15. Gu YH, Kodama H, Murata Y *et al*. ATP7A gene mutations in 16 patients with Menkes' disease and a patient with occipital horn syndrome. *Am J Med Genet* 2001; **99**: 217.
16. Kaler SG. Menkes disease. *Adv Pediatr* 1994; **41**: 263.
17. Kaler SG. Diagnosis and therapy of Menkes syndrome a genetic form of copper deficiency. *Am J Clin Nutr* 1998; **67**(Suppl.): 1029.
18. Adams PC, Strand RD, Bresnan MJ, Lucky AW. Kinky hair syndrome: serial study of radiological findings with emphasis on similarity to the battered child syndrome. *Radiology* 1974; **112**: 401.

19. Kodama H, Sato E, Yanagawa Y *et al*. Biochemical indicator for evaluation of connective tissue abnormalities in Menkes' disease. *J Pediatr* 2003; **142**: 726.
20. Seay AR, Bray PF, Wing SD *et al*. CT scans in Menkes disease. *Neurology* 1979; **29**: 304.
21. Wesenberg RL, Gwinn JL, Barnes GR. Radiological findings in the kinky hair syndrome. *Radiology* 1969; **92**: 500.
22. Blaser SI, Berns DH, Ross JS *et al*. Serial MR studies in Menkes disease. *J Comput Assist Tomogr* 1989; **13**: 113.
23. Oakes BW, Danks DM, Campbell PE. Human copper deficiency: ultrastructural studies of the aorta and skin in a child with Menke's syndrome. *Exp Mol Pathol* 1976; **25**: 82.
24. Danks DM, Cartwright E, Campbell PE, Mayne V. Is Menkes' syndrome a heritable disorder of connective tissue? *Lancet* 1971; **2**: 1089.
25. Gerdes AM, Tonnesen T, Pergament E *et al*. Variability in clinical expression of Menkes syndrome. *Eur J Pediatr* 1988; **148**: 132.
26. Kaler SG, Westman JA, Bernes SM *et al*. Gastrointestinal hemorrhage associated with gastric polyps in Menkes disease. *J Pediatr* 1993; **122**: 93.
27. Procopis P, Camakaris J, Danks DM. A mild form of Menkes' syndrome. *J Pediatr* 1981; **98**: 97.
28. Danks DM. The mild form of Menkes disease: progress report on the original case. *Am J Med Genet* 1988; **30**: 859.
29. Westman JA, Richardson DC, Rennert OM, Morrow G. Atypical Menkes steel hair disease. *Am J Med Genet* 1980; **30**: 1280.
30. Byers PH, Siegel RC, Holbrook KA *et al*. X-linked cutis laxa: defective cross-link formation in collagen due to decreased lysyl oxidase activity. *N Engl J Med* 1980; **303**: 61.
31. Peltonen L, Kuivaniemi H, Palotie A *et al*. Alterations of copper and collagen metabolism in the Menkes syndrome and a new subtype of Ehlers-Danlos syndrome. *Biochemistry* 1983; **22**: 6156.
32. Kuivaniemi H, Peltonen L, Palotie A *et al*. Abnormal copper metabolism and deficient lysyl oxidase activity in a heritable connective tissue disorder. *J Clin Invest* 1985; **69**: 798.
33. Kuivaniemi H, Peltonen L, Kivrikko KI. Type 1X Ehlers-Danlos syndrome and Menkes syndrome: the decrease in lysyl oxidase activity is associated with a corresponding deficiency in the enzyme protein. *Am J Hum Genet* 1985; **37**: 798.
34. Sartoris DJ, Luzzatti L, Weaver DD *et al*. Type 1X Ehlers-Danlos syndrome: a new variant with pathognomic radiographic features. *Radiology* 1984; **152**: 665.
35. Proud VK, Mussell HG, Kaler SG *et al*. Distinctive Menkes disease variant with occipital horns: delineation of natural history and clinical phenotype. *Am J Med Genet* 1996; **65**: 44.
36. Willemse J, Van Den Hamer CJ, Prins HW, Jonker PL. Menkes' kinky hair disease. I Comparison of classical and unusual clinical and biochemical features in two patients. *Brain Dev* 1982; **4**: 105.
37. Haas RH, Robinson A, Evans K *et al*. An X-linked disease of the nervous system with disordered copper metabolism and features differing from Menkes' disease. *Neurology* 1982; **31**: 852.
38. Mehes K, Petrovicz E. Familial benign copper deficiency. *Arch Dis Child* 1982; **57**: 716.
39. Goto S, Hirano A, Rojas-Corona RR. A comparative immunocytochemical study of human cerebellar cortex in X-chromosome-linked copper malabsorption (Menkes' kinky hair disease) and granule cell type degeneration. *Neuropathol Appl Neurobiol* 1989; **15**: 419.
40. Vuia O, Heye D. Neuropathologic aspects in Menkes' kinky hair disease (trichopoliodystrophy). *Neuropediatrics* 1974; **5**: 329.
41. Seelenfreud MH, Gartner S, Vinger PF. The ocular pathology of Menkes' disease. *Arch Ophthalmol* 1968; **80**: 718.
42. Morgello S, Peterson HD, Kahn LJ, Laufer H. Menkes kinky hair disease with 'ragged red' fibers. *Dev Med Child Neurol* 1988; **30**: 812.
43. Tonnesen T, Kleijer WJ, Horn N. Incidence of Menkes disease. *Hum Genet* 1991; **86**: 408.
44. Danks DM. Of mice and men, metals and mutations. *J Med Genet* 1986; **23**: 99.
45. Danks DM. Copper transport and utilisation in Menkes' syndrome and in mottled mice. *Inorg Perspect Biol Med* 1977; **1**: 73.
46. Hunt DM. Primary defect in copper transport underlines mottled mutants in the mouse. *Nature* 1974; **249**: 852.
47. Phillips M, Camakaris J, Danks DM. A comparison of phenotype and copper distribution in blotchy and brindled mutant mice and in nutritionally copper-deficient controls. *Biol Trace Elem Res* 1991; **29**: 11.
48. Brown RM, Camakaris J, Danks DM. Observation on the Menkes and brindled mouse phenotypes in cell hybrids. *Somat Cell Mol Genet* 1984; **10**: 321.
49. Murata Y, Kodama H, Abe T *et al*. Mutation analysis and expression of the mottled gene in the macular mouse model of Menkes disease. *Pediatr Res* 1997; **42**: 436.
50. Tümer Z, Vural B, Tønnesen T *et al*. Characterization of the exon structure of the Menkes disease gene using vectorette PCR. *Genomics* 1995; **26**: 437.
51. Dierick HA, Ambrosini L, Spencer J *et al*. Molecular structure of the Menkes disease gene (ATP7A). *Genomics* 1995; **28**: 462.
52. Silver S, Nucifora G, Chu L, Misra PK. Bacterial resistance ATPases: primary pumps for exporting toxic cations and anions. *Trends Biochem Sci* 1989; **14**: 76.
53. Levinson B, Gitschier J, Vulpe C *et al*. Are X-linked cutis laxa and Menkes disease allelic? *Nat Genet* 1993; **3**: 6.
54. Das S, Levinson B, Vulpe C *et al*. Similar splicing mutations of the Menkes/mottled copper-transporting ATPase gene in occipital horn syndrome and the blotchy mouse. *Am J Hum Genet* 1995; **56**: 570.
55. Camakaris J, Danks DM, Ackland L *et al*. Altered copper metabolism in cultured cells from human Menkes' syndrome and mottled mouse mutants. *Biochem Genet* 1980; **18**: 117.
56. Beratis NG, Price P, LaBadie G, Hirschorn K. ⁶⁴Cu metabolism in Menkes' and normal cultured skin fibroblasts. *Pediatr Res* 1978; **12**: 699.
57. Goka TJ, Stevenson RE, Hefferman PM, Howell RR. Menkes disease: a biochemical abnormality in cultured human fibroblasts. *Proc Natl Acad Sci USA* 1976; **73**: 604.
58. Yamaguchi Y, Heiny ME, Suzuki M, Gitlin JD. Biochemical characterization and intracellular localization of the Menkes disease protein. *Proc Natl Acad Sci USA* 1996; **93**: 14030.

59. Dierick HA, Adam AN, Escara-Wilke JF, Glover TW. Immunocytochemical localization of the Menkes copper transport protein (ATP7A) to the *trans* Golgi network. *Hum Mol Genet* 1997; **6**: 409.
60. Petris MJ, Mercer JFB, Culvenor JG *et al*. Ligand-regulated transport of the Menkes copper P-type ATPase efflux pump from the Golgi apparatus to the plasma membrane: a novel mechanism of regulated trafficking. *EMBO J* 1996; **15**: 6084.
61. Horn N. Menkes' X-linked disease: heterozygous phenotypic uncloned fibroblast cultures. *J Med Genet* 1980; **17**: 257.
62. Horn N, Mooy P, Berry C. Menkes X-linked disease. Two clonal cell populations in heterozygotes. *Clin Genet* 1986; **29**: 258.
63. Moore CM, Howell RR. Ectodermal manifestations in Menkes disease. *Clin Genet* 1985; **28**: 532.
64. Volpintesta EJ. Menkes' kinky hair syndrome in a black infant. *Am J Dis Child* 1974; **128**: 244.
65. Iwakawa Y, Niwa T, Tomita J. Menkes' kinky hair syndrome; report on an autopsy case and his female sibling with similar clinical manifestations. *Brain Dev* 1979; **11**: 260.
66. Barton NW, Dambrosia JM, Barranger JA. Menkes kinky-hair syndrome: report of a case in a female infant. *Neurology* 1983; **33**: 154.
67. Gerdes AM, Tonnesen T, Horn N *et al*. Clinical expression of Menkes syndrome in females. *Clin Genet* 1990; **38**: 452.
68. Kapur S, Higgins JV, Delp K, Rogers B. Menkes syndrome in a girl with X-autosome translocation. *Am J Med Genet* 1987; **26**: 503.
69. Verga V, Hall BK, Wang S *et al*. Localization of the translocation breakpoint in a female with Menkes syndrome to Xq132-q133 proximal to PGK-1. *Am J Hum Genet* 1991; **48**: 1133.
70. Tonnesen T, Horn N. Prenatal and postnatal diagnosis of Menkes disease an inherited disorder of copper metabolism. *J Inherit Metab Dis* 1989; **12**: 207.
71. Danks DM, Cartwright E, Stevens BJ, Townley RRW. Menkes kinky hair disease: further definition of the defect in copper transport. *Science* 1973; **179**: 1140.
72. Horn N. Menkes' X-linked disease: prenatal diagnosis of hemizygous males and heterozygous female. *Prenat Diagn* 1981; **1**: 107.
73. Das S, Whitney S, Taylor J *et al*. Prenatal diagnosis of Menkes disease by mutation analysis. *J Inherit Metab Dis* 1995; **18**: 364.
74. Tümer Z, Tønnesen T, Böhamann J *et al*. First trimester prenatal diagnosis of Menkes disease by DNA analysis. *J Med Genet* 1994; **31**: 615.
75. Rowe DW, McGoodwin EB, Martin GR, Grahn D. Decreased lysyloxidase activity in the aneurysm-prone mottled mouse. *J Biol Chem* 1977; **252**: 939.
76. Royce PM, Camakaris J, Danks DM. Reduced lysyl oxidase activity in skin fibroblasts from patients with Menkes' syndrome. *Biochem J* 1980; **192**: 579.
77. Holstein TJ, Fung RQ, Quevedo WC, Bienieki TC. Effect of altered copper metabolism induced by mottled alleles and diet on mouse tyrosinase. *Proc Soc Exp Biol Med* 1979; **162**: 264.
78. Kodama H, Okabe I, Yanagisawa M, Kodama Y. Copper deficiency in the mitochondria of cultured skin fibroblasts from patients with Menkes syndrome. *J Inherit Metab Dis* 1989; **12**: 386.
79. Williams DM, Atkin CL, Frens DB, Bray PF. Menkes' kinky hair syndrome: studies of copper metabolism and long-term copper therapy. *Pediatr Res* 1977; **11**: 823.
80. Sherwood G, Sarkar B, Sass Kortsak A. Copper histidinate therapy in Menkes' disease: prevention of progressive neurodegeneration. *J Inherit Metab Dis* 1989; **12**: 393.
81. Bidudhendra S, Lingertat-Walsh K, Clarke JTR. Copper-histidine therapy for Menkes disease. *J Pediatr* 1993; **123**: 828.
82. Kaler SG, Miller RC, Wolf EJ *et al*. *In utero* treatment of Menkes disease. *Pediatr Res* 1993; **33**: 192A.
83. Kanumakala S, Boneh A, Zacharin M. Pamidronate treatment improves bone mineral density in children with Menkes disease. *J Inherit Metab Dis* 2002; **25**: 391.

MUCOPOLYSACCHARIDOSES

75.	Introduction to mucopolysaccharidoses	555
76.	Hurler disease/mucopolysaccharidosis type IH/ α -L-iduronidase deficiency	558
77.	Scheie and Hurler-Scheie diseases/mucopolysaccharidosis IS and IHS/ α -iduronidase deficiency	566
78.	Hunter disease/mucopolysaccharidosis type II/iduronate sulfatase deficiency	572
79.	Sanfilippo disease/mucopolysaccharidosis type III	580
80.	Morquio syndrome/mucopolysaccharidosis type IV/keratan sulfatase deficiency	588
81.	Maroteaux-Lamy disease/mucopolysaccharidosis VI/N-acetylgalactosamine-4-sulfatase deficiency	597
82.	Sly disease/ β -glucuronidase deficiency/mucopolysaccharidosis VII	605

Introduction to mucopolysaccharidoses

The mucopolysaccharidoses are genetically determined disorders in which acid mucopolysaccharides, known chemically as glycosaminoglycans, are stored in the tissues [1, 2] and excreted in large quantities in the urine [3]. Storage in tissues leads to effects on a wide variety of systems and to remarkable changes in morphogenesis. Among these striking effects are the alterations in the appearance of the patient that are classically represented in the Hurler syndrome ([Chapter 76](#)). The elucidation of these disorders has provided clear evidence that even bizarre dysmorphic changes can be caused by single gene defects that interfere with body chemistry. They provide important models of the interaction of structure and function in humans. Impaired mental development and early demise, prior to 10 years of age in the Hurler syndrome, are the most devastating consequences of mucopolysaccharide accumulation in the central nervous and cardiovascular systems. However, there is considerable variety of expression among patients with various individual mucopolysaccharidoses. Patients with some syndromes are intellectually normal, and some survive well into adult life. Research in this field has proceeded rapidly, so that it is now possible to delineate the molecular defect at the level of the enzyme in each of the mucopolysaccharidoses, and the genes of most of them have been cloned and mutations in them identified.

Advances in the understanding of the mucopolysaccharidoses followed the growth of fibroblasts from these patients in cell culture and the recognition that there was phenotypic expression of the disease in the fibroblast. The elucidation of the molecular nature of the mucopolysaccharidoses represents a fascinating chapter in cell biology. Characterization of the mucopolysaccharidoses as disorders in the degradation of intracellular acid mucopolysaccharide began with the studies of *Fratantoni et al.* [4] using ^{35}S -labeled sulfate. $^{35}\text{SO}_4$ is taken up by the cells of patients, just as it is by normal cells. However, in patients as opposed to controls, there is no turnover; these cells simply accumulate the label and keep it.

In what is now a landmark series of experiments, *Fratantoni et al.* [5] mixed normal fibroblasts in culture with those of patients with Hurler or Hunter syndrome and found that the kinetics of $^{35}\text{SO}_4$ incorporation became normal. Furthermore, it was possible to restore normal

kinetics in Hurler cells by mixing them with Hunter cells and vice versa. It was also found that the medium in which normal cells or Hunter cells had grown could correct the defect in Hurler cells. Corrective factors were soon identified for other mucopolysaccharidoses [6]. In fact, demonstration of two different corrective factors first permitted the distinction of Sanfilippo types A and B. On the other hand, fibroblasts from patients with Scheie syndrome could not be corrected by the factor from Hurler cells [7], indicating that the genes for these two conditions were allelic and represented different defects in the same enzyme protein. These studies in cell biology led directly to the identification of the enzymatic defects [8, 9] ([Table 75.1](#)). They also laid the groundwork for current enzyme replacement therapy with recombinant enzymes.

Hurler disease was originally classified by McKusick as mucopolysaccharidosis type I [1]. With the recognition of the enzyme defect in α -L-iduronidase and the fact that defective activity of the same enzyme was also the cause of the Scheie syndrome [8], the subclassifications IH for Hurler and IS for Scheie were employed. The classification of the mucopolysaccharidoses and a summary of their clinical biochemical characteristics are shown in [Table 75.1](#). The seven types of mucopolysaccharidosis represent the deficiencies of 11 specific enzymes. Prenatal diagnosis was initially carried out in Hurler and Hunter diseases by measuring labeled sulfate incorporation in cultured amniocytes [10].

The defect in the Hurler cell is in α -L-iduronidase [8, 9, 11] (see [Figure 76.1](#)), and Hurler corrective factor has been shown to have iduronidase activity [8]. The Hurler corrective factor is a form of iduronidase that can be taken up by fibroblasts [12] because it contains the mannose-6-phosphate recognition marker, whereas the lower molecular weight enzyme purified from human kidney cannot. The mucopolysaccharidoses I–VII represent defective activity in the enzymes required for the stepwise degradation of heparan sulfate, dermatan sulfate, keratan sulfate, or chondroitin sulfate. The gene and the cDNAs for the enzymes defective in the mucopolysaccharidoses with the exception of the MPS IIIC enzyme have been mapped to their respective chromosomes [13] and cloned [14], and many mutations have been identified. In the absence of

Table 75.1 Clinical and laboratory characteristics of the mucopolysaccharidoses

Syndrome	MPS designation ^a	Inheritance	Impaired mental development	Corneal clouding	Hepatosplenomegaly	Skeletal defect	Other clinical manifestations	Glycosaminoglycans stored excreted	Defective enzyme
Hurler	I _H	Autosomal recessive	+	+	+	+	Coarse facial features, cardiac disease, motor weakness, hernia	Dermatan sulfate heparan sulfate	α -L-iduronidase
Scheie	I _S	Autosomal recessive	—	+	—	+	Coarse features, stiff joints	Dermatan sulfate heparan sulfate	α -L-iduronidase
Hurler/Scheie	I _{HS}	Autosomal recessive	±	+	+	+	Phenotype intermediate	Dermatan sulfate heparan sulfate	α -L-iduronidase
Hunter	II	X-linked recessive	+	—	+	+	Coarse features, weakness, aggressive behavior	Dermatan sulfate heparan sulfate	Iduronate sulfatase
Sanfilippo Type A	III _A	Autosomal recessive	+	—	±	+	Mild somatic features, contrast with severity of cerebral disease	Heparan sulfate	Heparan N-sulfatase (sulfamidase)
Sanfilippo Type B	III _B	Autosomal recessive	+	—	±	+	Mild somatic features, contrast with severity of cerebral disease	Heparan sulfate	α -N-Acetylglucosaminidase
Sanfilippo Type C	III _C	Autosomal recessive	+	—	±	+	Mild somatic features, contrast with severity of cerebral disease	Heparan sulfate	AcetylCoA: α -D-glucosaminide- <i>N</i> -acetyl transferase
Sanfilippo Type D	III _D	Autosomal recessive	+	—	±	+	Mild somatic features, contrast with severity of cerebral disease	Heparan sulfate	<i>N</i> -Acetyl- α -D-Type D glucosaminide-6-sulfatase
Morquio A	IV _A	Autosomal recessive	±	+	—	+	Distinctive bone deformities, hypoplastic odontoid, thin enamel	Keratan sulfate	Galactose-6-sulfatase
Morquio B	IV _B	Autosomal recessive	±	+	+	+	Mild bone changes, hypoplastic odontoid	Keratan sulfate	β -Galactosidase
Maroteaux-Lamy	VI	Autosomal recessive	—	+	+	+	Severe bony deformities, valvular cardiac disease	Dermatan sulfate	Acetylglucosamine 4-sulfatase (arylsulfatase β)
Sly	VII	Autosomal recessive	+	+	+	+	Coarse features	Dermatan sulfate heparan sulfate, chondroitin-4-6-sulfates	β -Glucuronidase
	IX	Autosomal recessive	+	—	—	+	Periarticular soft tissue masses; short stature	Hyaluronan	Hyaluronidase

Types V and VIII have become obsolete.

effective enzyme activity the glycosaminoglycans are stored in the lysosomes.

A suspected diagnosis of mucopolysaccharidoses is often pursued chemically by the documentation of increased amounts of mucopolysaccharide in the urine. However, spot tests for mucopolysaccharide are unreliable and give false positive and negative results [15]. Semiquantitative and quantitative procedures may also be misleading [16]. If a diagnosis of a mucopolysaccharidoses is suspected, assay of the lysosomal enzymes should be performed. This is readily carried out in freshly isolated leukocytes. It can also be done on cultured fibroblasts.

A common feature among the mucopolysaccharidoses is the roentgenographic appearance [17, 18] known as dysostosis multiplex. This picture is best exemplified in Hurler disease. This is such a constant feature of the disease that roentgenographic search for the presence of dysostosis multiplex is an effective way to screen for the mucopolysaccharidoses. It is reliable in all but the Sanfilippo patients, and it is most dramatic in the Hurler patients. This picture is also seen in generalized GM₁ gangliosidosis (Chapter 89) and in the mucopolipidoses (Chapters 83 and 84). It is described in detail in Chapter 76.

Therapy has been successful via bone marrow transplantation in some of these diseases. Enzyme replacement therapy has been of mixed efficacy, but certainly there are effects on some features of the diseases. A variety of supportive measures, such as surgical fusion to stabilize a hypoplastic odontoid, can be of great benefit.

REFERENCES

- McKusick VA. *Heritable Disorders of Connective Tissue*, 4th edn. St Louis: CV Mosby Co, 1972: 521.
- Brante G. Gargoylism. A mucopolysaccharidosis. *Scand J Clin Lab Invest* 1952; **4**:43.
- Dorfman A, Lorincz AE. Occurrence of urinary mucopolysaccharides in the Hurler syndrome. *Proc Natl Acad Sci USA* 1957; **43**:443.
- Fratantoni JC, Hall CW, Neufeld EF. The defect in Hurler's and Hunter's syndromes: faulty degradation of mucopolysaccharides. *Proc Natl Acad Sci USA* 1968; **60**: 699.
- Fratantoni JC, Hall CW, Neufeld EF. Hurler and Hunter syndromes. Mutual correction of the defect in cultured fibroblasts. *Science* 1968; **162**: 570.
- Neufeld EF, Cantz MJ. Corrective factors for inborn errors of mucopolysaccharide metabolism. *Ann NY Acad Sci* 1971; **179**: 580.
- Wiesmann U, Neufeld EF, Scheie and Hurler syndromes. Apparent identity of the biochemical defect. *Science* 1970; **169**: 72.
- Bach G, Friedman R, Weismann B, Neufeld EF. The defect in the Hurler and Scheie syndromes: deficiency of α -L-iduronidase. *Proc Natl Acad Sci USA* 1972; **69**: 2048.
- Matalon R, Cifonelli JA, Dorfman A. L-iduronidase in cultured human fibroblasts and liver. *Biochem Biophys Res Commun* 1971; **42**: 340.
- Fratantoni JC, Neufeld EF, Uhlendorf BW, Jacobson CB. Intrauterine diagnosis of the Hurler and Hunter syndrome. *N Engl J Med* 1969; **280**: 686.
- Matalon R, Dorfman A. Hurler's syndrome, an α -L-iduronidase deficiency. *Biochem Biophys Res Commun* 1972; **47**: 959.
- Shapiro LJ, Hall CE, Leder IG, Neufeld EF. The relationship of α -L-iduronidase and Hurler corrective factor. *Arch Biochem Biophys* 1976; **172**: 156.
- Scott HS, Ashton LJ, Eyre HJ *et al*. Chromosomal localization of the human α -L-iduronidase gene (IDUA) to 4p163. *Am J Hum Genet* 1990; **47**: 802.
- Scott HS, Guo X-H, Hopwood JJ, Morris CP. Structure and sequence of the human α -L-iduronidase gene. *Genomics* 1992; **13**: 1811.
- De Jong JGN, Hasselman JJF, van Landeghem AAJ *et al*. The spot test is not a reliable screening procedure for mucopolysaccharidoses. *Clin Chem* 1991; **37**: 572.
- Thuy LP, Nyhan WL. A new quantitative assay for glycosaminoglycans. *Clin Chim Acta* 1992; **212**: 17.
- Caffey J. Gargoylism (Hunter-Hurler disease, dysostosis multiplex, lipochondrodystrophy). *Am J Roentgenol Radium Ther Nucl Med* 1952; **67**: 715.
- Grossman H, Dorst JP. The mucopolysaccharidoses and mucopolipidoses. In: Kauffman HJ (ed.). *Progress in Pediatric Radiology*. Basel: Charger, 1973: 495.

Hurler disease/mucopolysaccharidosis type IH/ α -L-iduronidase deficiency

Introduction	558	Treatment	563
Clinical abnormalities	558	References	564
Genetics and pathogenesis	563		

MAJOR PHENOTYPIC EXPRESSION

Coarse features, impaired mental development, corneal clouding, hepatosplenomegaly, short stature, dysostosis multiplex, and cardiac complications; widespread lysosomal storage of mucopolysaccharide, and excretion of dermatan sulfate and heparan sulfate; and deficiency of α -L-iduronidase.

INTRODUCTION

The Hurler syndrome is the classic or prototypic mucopolysaccharidosis (MPS). Hurler's original description was published in 1919 [1]. McKusick classified it as mucopolysaccharidosis I [2], and more recently as IH to distinguish it from the Scheie phenotype IS, or the intermediate Hurler-Scheie (IHS) picture. Modern molecular biology makes these distinctions less relevant, but we have continued to separate mucopolysaccharidosis I into two chapters because of the importance of these

phenotypes and because these distinctions may have relevance to therapy.

The defect in the Hurler cell is in α -L-iduronidase (Figure 76.1) [3–5]. The gene for α -L-iduronidase has been mapped to chromosome 4p16.3 [6] and has been cloned and sequenced [7]. A number of mutations has been identified, including at least two common alleles, W402X and Q70X, accounting for over half the alleles in European patients [8–10]. Heterogeneity is also evident in different mutations in other ethnic groups [11, 12].

CLINICAL ABNORMALITIES

Patients with Hurler syndrome appear normal at birth. They develop normally for some months, after which they begin to develop progressive disease. Patients may present first for repair of inguinal hernias or for chronic rhinitis [13]. The diagnosis is seldom suspected at that time. However, as the first year of life proceeds, the characteristic appearance develops. Nasal discharge tends to be persistent, as are recurrent respiratory infections and otitis. Breathing is noisy, as is snoring.

In the established syndrome the facial features are coarse (Figures 76.2, 76.3, 76.4, 76.5, and 76.6). The head is large, bulging, and scaphocephalic, and there may be hyperostosis of the sagittal sutures. Frontal bossing, prominent brow, wide-set prominent eyes with puffy-appearing lids, and a

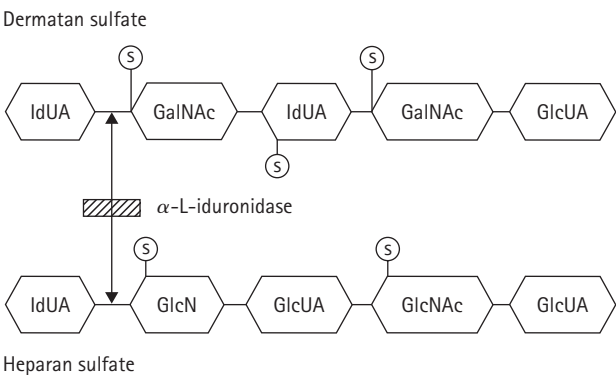


Figure 76.1 α -L-iduronidase, the site of the defect in Hurler and Scheie diseases. Dermatan sulfate and heparan sulfate accumulate when α -L-iduronidase activity is defective.



Figure 76.2 DD: A six-year-old girl with Hurler disease. She was short (90 cm) at the age of seven years and had a relatively large head (55 cm). The facial features were coarse, the eyes were prominent, and the nasal bridge depressed. There was frontal bossing. The abdomen was protuberant because of hepatosplenomegaly, and there was an umbilical hernia.

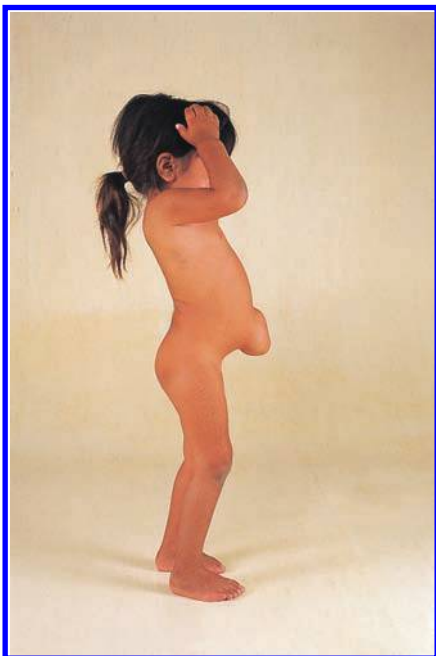


Figure 76.3 MOMMR: This toddler with Hurler disease illustrates the evolution of the disease. The abdomen was protuberant and facial features coarse, but much less than in Figure 76.2.



Figure 76.4 SC: This child, at two years and ten months, with α -iduronidase deficiency had clearcut dysostosis multiplex, and her hand was the typical claw hand, but her facial features were subtle. The alae nasi and septum had begun to widen, and she was quite hirsute; corneas had begun to cloud.



Figure 76.5 BR: This five-year-old girl with advanced Hurler disease had massive hepatosplenomegaly and a gibbus.

depressed nasal bridge are characteristic. The face is flat, and the nose and nostrils wide and anteverted (Figures 76.2, 76.5, and 76.6). The lips are large and thickened; the tongue is large and often protrudes through the open mouth (see Figure 77.11). There is hypertrophy of the gums and the bony alveolar ridges; the teeth are small and widely spaced. Patients generally have hirsutism. The hair



Figure 76.6 GAN: An infant with pronounced stigmata of Hurler disease. The facial features were quite coarse as early as five months. Activity of α -L-iduronidase was undetectable.



Figure 76.7 DD: The corneas were steamy.

is thick and coarse, the eyebrows bushy and the hairline low, and there is a large amount of forehead hair. Lanugo hair is plentiful. The skin is thick.

Clouding of the cornea is a hallmark of the syndrome (Figure 76.7). The cloudy cornea has a ground-glass appearance. It may lead to blindness. Nystagmus and strabismus are occasionally seen. Some patients develop glaucoma [14]. Sensorineural or mixed conductive and neural deafness develops regularly.

Developmental delay may be evident within the first 12 months, but intellectual deterioration is progressive to a level of severe impairment. The peak of intellectual function may be about two to four years of age or earlier, after which there is a steady regression. Behavior is usually

quite pleasant, and these are often lovable children despite their unusual appearance.

Shortness of stature is characteristic. Linear growth appears to stop at two to three years of age. Maximum height in one large series was 97 cm [13]; few exceed 100 cm. The neck is short, and the large head appears to rest directly on the thorax. The lower rib cage flares. The back is kyphotic, and there is a gibbus in the lower thoracic or upper lumbar area (Figure 76.5). The joints become stiff, and mobility may be severely limited, especially at the elbows. The hands become broad, and the fingers stubby. This, and the limitation of extension and the position in flexion, produces the characteristic claw hand (Figure 76.8). The abdomen is protuberant. The liver and spleen become very large and very hard. Umbilical hernias are the rule, and inguinal hernias and hydroceles are common. Recurrence of a hernia is frequent following surgical repair.

Cardiac complications are prominent late features of the disease and often represent the cause of death. Some patients have been reported in whom acute cardiomyopathy and endocardial fibroelastosis were evident in the first year of life [15, 16]. These are infantile cardiac manifestations. Later cardiac disease is valvular; murmurs, aortic regurgitation, and mitral or tricuspid atresia result from storage of mucopolysaccharide in the valves. These features lead to congestive cardiac failure. Thickening of the valves of the coronary arteries leads to angina pectoris and myocardial infarction. Coronary angiography may underestimate the degree of involvement [17]. Patients may also die of pneumonia. They tolerate anesthesia very poorly [13].

The roentgenographic appearance of dysostosis multiplex in these patients is classic (Figures 76.9, 76.10, 76.11, 76.12, 76.13, and 76.14) [18, 19]. The shafts of all of the bones widen. The cortical walls become thickened externally during the first year of life, but later they become thin as the medullary cavity dilates. Lack of normal modeling and tubulation characterizes all of the bones (Figures 76.9 and 76.10). Epiphyseal centers are



Figure 76.8 The typical claw hands of a patient with Hurler disease. Limitation of motion is evident in the position of the digits.



Figure 76.9 DD: Dysostosis multiplex is seen classically in the bones of the hand. The radial and ulnar articular surfaces are angulated toward each other. Marked irregularity and impaired ossification of the carpal bones are seen, as well as coarsening of the trabeculae of the phalanges and metacarpals. The metacarpals are broadened at their distal ends and tapered at the proximal ends with a hook-like deformity. The phalanges, especially the distal ones, are short and the proximal and middle phalanges are characteristically thick and bullet-shaped.

poorly developed. The bones of the upper extremities become short and stubby (Figure 76.10) and taper toward the ends, often with enlargement of the mid-portions. The ends of the radius and ulna angulate toward each other (Figure 76.10). The roentgenographic appearance of the claw hand (Figure 76.9) of the patient with Hurler syndrome is pathognomonic of dysostosis multiplex. The metacarpals are broad at their distal ends and taper at their proximal ends. The phalanges are thickened and bullet-shaped. The lower extremities show moderate enlargement of the shaft. There may be coxa valga, small femoral heads, and a poorly developed pelvis. The lower ribs are broad and spatulate (Figure 76.11). The clavicle is absolutely characteristic, while the lateral portion may be hypoplastic or even absent. The vertebrae are hypoplastic, scalloped posteriorly and beaked anteriorly, especially at the thoracolumbar junction (Figure 76.12). In this area, there is anterior vertebral wedging, and this leads to the thoracolumbar gibbus, with typically a hooked-shaped vertebra at the gibbus. Hypoplasia of the odontoid may be present, and this can lead to atlantoaxial subluxation, as in Morquio disease (Chapter 81). The skull is large, the orbits shallow, and the sella turcica shoe-shaped or J-shaped (Figures 76.13 and 76.14).



Figure 76.10 DD: The long bones of the upper extremity illustrate the lack of normal modeling and tubulation of the diaphyses, making these bones short and stubby. There was a varus deformity of the humerus. The ulnar semilunar notch was shallow and the radioulnar inclination abnormal.



Figure 76.11 DD: The roentgenographic appearance of the ribs was classic. The spatulate shape is caused by a generalized widening of the ribs, which spares the relatively narrow proximal portions.



Figure 76.12 Roentgenographic appearance of the spine of DD. The antero-posterior distance was diminished in the vertebral bodies, and there was marked posterior scalloping. The pedicles of the lumbar spine were elongated. There was a marked thoracolumbar gibbus and inferior beaking of T12, L1, and L2.

Complications include cord compression, hydrocephalus, and pigmentary degeneration of the retina. Death usually occurs by ten years of age. At autopsy, the weight of the brain is increased, indicating that the increase in head size is a consequence of the storage of material. Thickening of the meninges is also seen. It is for this reason that some patients develop hydrocephalus. Pachymeningitis in the cervical area may also lead to myelitis or spinal nerve root compression. Electron-microscopic examination of the brain reveals the presence of zebra bodies resembling those of Tay-Sachs disease ([Chapter 91](#)). These findings have been interpreted as indicating the accumulation of ganglioside in brain [20], and this has been documented chemically [21]. Large vacuolated cells are found in many tissues.

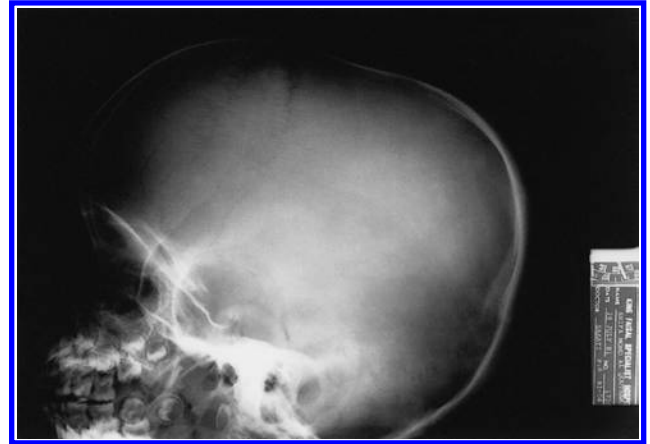


Figure 76.13 SMQ: Roentgenogram of the skull illustrates the early appearance of the J-shaped sella turcica.



Figure 76.14 Roentgenographic appearance of the skull of DD. The very large cranium of both occipital and frontal areas was prominent. There was calcification of the choroid plexus of the left lateral ventricle. There was enormous enlargement of the sella and erosion of the clinoid processes. The mandibular rami were short, and there was increased angulation at the junction of the body and the ramus, as well as flattening of the condyles.

Characteristic granules (Reilly bodies) are found in the polymorphonuclear and other leukocytes ([Figure 76.15](#)). The mucopolysaccharide found in tissues such as the liver and spleen is dermatan sulfate [21, 22]. Large quantities of dermatan sulfate and heparan sulfate are excreted in the urine. In Hurler syndrome, these two compounds are excreted in an approximate ratio of 2:1. Mucopolysaccharide also accumulates in the brain. Metachromasia may be demonstrated in cultured fibroblasts by a pink stain with toluidine blue [23]. Quantitative analyses have revealed increased amounts of dermatan sulfate in fibroblasts of patients with Hurler syndrome [24, 25].

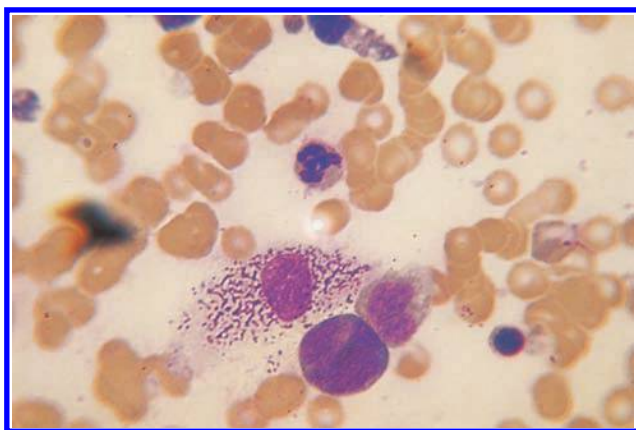


Figure 76.15 Bone marrow illustrating the Reilly bodies of Hurler syndrome. The histiocytes in the center of the field were full of these inclusions. (This illustration was kindly provided by Dr Faith Kung of the University of California San Diego.)

GENETICS AND PATHOGENESIS

The Hurler syndrome is determined by an autosomal recessive gene. Parental consanguinity has commonly been reported. The incidence of the disease in a British Columbia survey was estimated at one in 144,000 births [26].

The molecular defect in Hurler disease is in the activity of α -L-iduronidase (Figure 76.1) [3–5]. This enzyme catalyzes the hydrolysis terminal iduronic acid residues of dermatan sulfate and heparan sulfate. The enzyme has been purified from human liver, kidney, and lung [27–29]. The cDNA codes a protein of 653 amino acids [30]. The protein exists as a monomer of 70 kDa minus the signal sequence [31]. There are six potential sites for N-glycosylation. It acquires mannose-6-phosphate, which permits targeting to lysosomes [31]. The deficient activity of enzyme is readily demonstrated in cultured fibroblasts and in leukocytes [32–35]. Residual activity of the enzyme has not been useful in distinguishing variants with phenotypes of greater or lesser severity, including the Scheie syndrome. Immunochemical studies have also not been helpful with these distinctions.

Carrier detection can be performed by assay of the enzyme in cultured fibroblasts or in freshly isolated leukocytes, in either of which activity of iduronidase is about half that of normal cells [36, 37]. However, the ranges of activity in both normal and carrier populations are so great that it may be difficult to ascertain for certain that any individual is a non-carrier [38]. A positive identification of a carrier should be reliable. If the mutation in the proband is known, analysis of the DNA for heterozygosity in relatives is precise.

Prenatal diagnosis may be carried out by assay of ^{35}S -mucopolysaccharide accumulation or the activity of iduronidase [39]. In the ^{35}S assay, normal amniocytes behave just like fibroblasts, but iduronidase activity is much lower in amniocytes than in fibroblasts, and this

could cause difficulties in distinguishing a heterozygote from an affected fetus. Prenatal diagnosis has also been accomplished by assay of iduronidase in chorionic villi [40], but activity is normally so very low in this material that great care is required. Genetic diagnosis is preferable when feasible.

The gene for iduronidase consists of 14 exons and approximately 19 kb. A large 13-kb intron separates the second and third exons [7]. There is a canine model of MPSI, and the canine gene for iduronidase has the same structure as the human gene [41]. The mutation in the model is a G to A transition in the donor splice site of intron 1, which leads to retention of this large intron in the RNA and premature termination of the intron–exon junction. A locus D4S111 linked to Huntington disease on chromosome 4 has turned out to be the iduronidase gene [42].

The mutations that account for more than 50 percent of the alleles in populations of European origin change a tryptophan at position 402 and a glutamine at 70 to stop codons; both yield no detectable functional protein [8–10]. Premature termination is also present at a deletion/insertion in exon 6 for which a Libyan Jewish patient with Hurler disease was homozygous [11]. Stop codons resulting from changes of tyrosine 64 and glutamine 310 were found in Arab patients [12], as well as a threonine-to-proline change at 366 and a glycine-to-arginine change at 409. Among Japanese, there are two common mutations, a 5-bp insertion between nts704 and 705, and R89Q which is seen in Caucasians, but uncommonly [43]. Homozygotes for all of these mutations, except the Japanese missense mutation and compounds of any two of the others, have a severe Hurler phenotype. Homozygosity for the first of these Japanese mutations conveyed a severe phenotype. Splice site mutations and deletions have also been observed [44]. In addition to the considerable mutational heterogeneity among MPSI patients, there are many polymorphic alleles consistent with common haplotype structure [45]. In the homozygous setting, null mutations and coding disruptions lead to the severe Hurler phenotype [45]; missense mutations are found that are individually characteristic of the H, HS, or S phenotypes.

Newborn screening is feasible. Chamoles *et al.* [46] demonstrated that the enzyme is stable in dried blood spots, and an assay using tandem mass spectrometry has been developed [47].

TREATMENT

The discovery of the MPS correcting factor capable of correcting the defective glycosaminoglycan catabolism in cultured cells raised the hope that these diseases might be treatable by transplantation or enzyme replacement therapy. The availability of animal models and recombinant enzyme with the mannose-6-phosphate recognition signal [48], as well as successes in the clinical management of

Gaucher disease provided hope for successful enzyme replacement therapy. Recombinant iduronidase prepared in hamster cells administered to homozygous animals led to major improvement in storage in liver, spleen, and kidney, but no improvement in brain, heart valves, or cornea [48].

Enzyme replacement with human recombinant α -L-iduronidase was first reported [49, 50] in 45 patients with MPSI. Patients were selected with Hurler-Scheie or Scheie phenotypes and given enzyme intravenously weekly for as long as 62 weeks. Hepatosplenomegaly decreased significantly in all patients. Liver size was normal in eight patients by 26 weeks. Growth in height and weight increased in prepubertal patients. Improvements were also notable in the urinary excretion of glycosaminoglycans, as well as joint mobility, respiratory function, and ambulation (6 minute walk test). The enzyme was approved by the US Food and Drug Administration (FDA) in 2003 and is marketed as Aldurazyme (BioMarin/Genzyme). Corneal clouding does not change, and cardiac valvular disease seems to be unaltered. There is little likelihood that this approach would affect the brain; trials are underway to treat with intrathecal enzyme delivery [51].

Bone marrow transplantation [52] was followed by arrest or reversal of many of the peripheral features of the disease. It did not seem likely that this would appreciably affect the central nervous system, but longer-term follow up of the results of bone marrow transplantation in Hurler disease [53] have documented resolution of hydrocephalus and, in four patients with normal IQs before the procedure, maintenance of intelligence for two to seven years post-transplantation. It appears clear that if performed early enough, bone marrow transplantation will preserve cerebral function.

Magnetic resonance spectroscopy indicated high ratios of presumptive MPS to creatinine that did not fall after bone marrow transplantation [54]. Bone marrow transplantation appears not to improve the skeletal or ocular manifestations of the disease. In patients without a compatible donor, unrelated umbilical cord blood transplantation may be an option. It is possible that transplantation and enzyme replacement therapy may be complementary.

Supportive management includes shunting for hydrocephalus, surgical decompression for carpal tunnel syndrome and spinal stenosis, tonsillectomy and adenoidectomy for airway obstruction, management of otitis media, hearing aids, and visual aids. Inguinal hernias should be repaired. Cardiac valvular surgery may be indicated.

REFERENCES

1. Hurler G. Ueber einen Typ multipler Abartungen Vorwiegend am Skelettsystem. *Z Kinderheilk* 1919; **24**: 220.
2. McKusick VA. *Heritable Disorders of Connective Tissue*, 4th edn. St Louis: CV Mosby, 1972: 521.
3. Bach G, Friedman R, Weismann B, Neufeld EF. The defect in the Hurler and Scheie syndromes: deficiency of α -L-iduronidase. *Proc Natl Acad Sci USA* 1972; **69**: 2048.
4. Matalon R, Cifonelli JA, Dorfman A. L-Iduronidase in cultured human fibroblasts and liver. *Biochem Biophys Res Commun* 1971; **42**: 340.
5. Matalon R, Dorfman A. Hurler's syndrome, an α -L-iduronidase deficiency. *Biochem Biophys Res Commun* 1972; **47**: 959.
6. Scott HS, Ashton LJ, Eyre HJ *et al*. Chromosomal localization of the human α -L-iduronidase gene (IDUA) to 4 p 163. *Am J Hum Genet* 1990; **47**: 802.
7. Scott HS, Guo X-H, Hopwood JJ, Morris CP. Structure and sequence of the human α -L-iduronidase gene. *Genomics* 1992; **13**: 1811.
8. Bunge S, Kleijer WJ, Steglich C *et al*. Mucopolysaccharidosis type I: identification of 8 novel mutations and determination of the frequency of the two common alpha-L-iduronidase mutations (W402X and Q70X) among European patients. *Hum Mol Genet* 1994; **3**: 861.
9. Scott HS, Lijjens T, Hopwood JJ, Morris CP. A common mutation for mucopolysaccharidosis type 1 associated with a severe Hurler phenotype. *Hum Mutat* 1992; **1**: 103.
10. Scott HS, Litjens T, Nelson PV *et al*. α -L-Iduronidase mutations (Q70X and P533X) associated with severe Hurler phenotype. *Hum Mutat* 1992; **1**: 333.
11. Moskowitz SM, Tieu PT, Neufeld EF. A deletion/insertion mutation in the IDUA gene in a Libyan Jewish patient with Hurler syndrome (Mucopolysaccharidosis I). *Hum Mutat* 1993; **2**: 71.
12. Bach G, Moskowitz SM, Tieu PT, Neufeld EF. Molecular analysis of Hurler syndrome in Druze and Muslim Arab patients in Israel: multiple allelic mutations of the IDUA gene in a small geographic area. *Am J Hum Genet* 1993; **53**: 330.
13. Leroy JG, Crocker C. Clinical definition of the Hurler-Hunter phenotypes. A review of 50 patients. *Am J Dis Child* 1966; **112**: 518.
14. Nowaczyk MJ, Clarke JT, Morin JD. Glaucoma as an early complication of Hurler's disease. *Arch Dis Child* 1988; **63**: 1091.
15. Donaldson MDC, Pennock CA, Berry PJ *et al*. Hurler syndrome with cardiomyopathy in infancy. *J Pediatr* 1989; **114**: 430.
16. Stephan MJ, Stevens EL Jr, Wenstrup RJ *et al*. Mucopolysaccharidosis I presenting with endocardial fibroelastosis of infancy. *Am J Dis Child* 1989; **143**: 782.
17. Braunlin EA, Hunter DQ, Krivit W *et al*. Evaluation of coronary artery disease in the Hurler syndrome by angiography. *Am J Cardiol* 1992; **69**: 1487.
18. Caffey J. Gargoylism (Hunter-Hurler disease dysostosis multiplex lipochondrodystrophy). *Am J Roentgenol Radium Ther Nucl Med* 1952; **67**: 715.
19. Grossman H, Dorst JP. The mucopolysaccharidoses and mucopolipidoses. In Kauffman HJ (ed.). *Progress in Pediatric Radiology*. Basel: Karger, 1973: 495.
20. McKusick VA. The nosology of the mucopolysaccharidoses. *Am J Med* 1969; **47**: 730.
21. Dorfman A, Matalon R. The Hurler and Hunter syndromes. *Am J Med* 1969; **47**: 691.

22. Muir H. The structure and metabolism of mucopolysaccharides. *Am J Med* 1969; **47**: 673.
23. Danes BS, Bearn AG. Hurler's syndrome: demonstration of an inherited disorder of connective tissue in cell culture. *Science* 1965; **149**: 989.
24. Matalon R, Dorfman A. Acid mucopolysaccharides in cultured human fibroblasts. *Lancet* 1969; **2**: 838.
25. Matalon R, Dorfman A. Hurler's syndrome: biosynthesis of acid mucopolysaccharides in tissue culture. *Proc Natl Acad Sci USA* 1966; **56**: 1310.
26. Lowry RB, Renwick DHG. Relative frequency of the Hurler and Hunter syndromes. *N Engl J Med* 1971; **284**: 221.
27. Rome LH, Garvin AJ, Neufeld EF. Human kidney α -L-iduronidase: purification and characterization. *Arch Biochem Biophys* 1978; **189**: 344.
28. Clemens PR, Brooks DA, Saccone GPT, Hopwood JJ. Human α -L-iduronidase. 1 Purification monoclonal antibody production and subunit molecular mass. *Eur J Biochem* 1985; **152**: 21.
29. Schuchman EH, Guzman NA, Desnick RJ. Human α -L-iduronidase. 1 Purification and properties of the high uptake (higher molecular weight) and low uptake (processed) forms. *J Biol Chem* 1984; **259**: 3132.
30. Scott HS, Anson DS, Orsborn AM *et al*. Human α -L-iduronidase: cDNA isolation and expression. *Proc Natl Acad Sci USA* 1991; **88**: 9695.
31. Myerowitz R, Neufeld EF. Maturation of α -L-iduronidase in cultured human fibroblasts. *J Biol Chem* 1981; **256**: 3044.
32. Hall CW, Liebaers I, Di Natale P, Neufeld EF. Enzymic diagnosis of the genetic mucopolysaccharide storage disorders. *Meth Enzymol* 1978; **50**: 439.
33. Kresse H, von Figura K, Klein U *et al*. Enzymic diagnosis of the genetic mucopolysaccharide storage disorders. *Meth Enzymol* 1982; **83**: 559.
34. Hopwood JJ, Muller V, Smithson A, Baggett N. A fluorometric assay using 4-methylumbelliferyl α -L-iduronide for the estimation of α -L-iduronidase activity and the detection of Hurler and Scheie syndromes. *Clin Chim Acta* 1979; **92**: 257.
35. Weissmann B. Synthetic substrates for α -L-iduronidase. *Meth Enzymol* 1978; **50**: 141.
36. Hall CW, Neufeld EF. α -L-iduronidase activity in cultured skin fibroblasts and amniotic fluid cells. *Arch Biochem Biophys* 1973; **158**: 817.
37. Kelly TE, Taylor HA Jr. Leukocyte values of α -L-iduronidase in mucopolysaccharidosis I. *J Med Genet* 1976; **13**: 149.
38. Shapiro LJ. Current status and future direction for carrier detection in lysosomal storage diseases. In: Callahan JW, Lowden JA (eds). *Lysosomes and Lysosomal Storage Diseases*. New York: Raven Press, 1981: 343.
39. Frattantoni JC, Neufeld EF, Uhlendorf BW, Jacobson CB. Intrauterine diagnosis of the Hurler and Hunter syndrome. *N Engl J Med* 1969; **280**: 686.
40. Young EP. Prenatal diagnosis of Hurler disease by analysis of α -L-iduronidase in chorionic villi. *J Inherit Metab Dis* 1992; **15**: 224.
41. Menon KP, Tieu PT, Neufeld EF. Architecture of the canine IDUA gene and mutation underlying canine mucopolysaccharidosis. *Genomics* 1992; **14**: 763.
42. MacDonald ME, Scott HS, Whaley WL *et al*. Huntington disease-linked locus D4S111 exposed as the α -L-iduronidase gene. *Somat Cell Mol Genet* 1991; **17**: 421.
43. Yamagishi A, Tomatsu S, Fukuda S *et al*. Mucopolysaccharidosis type I: identification of common mutations that cause Hurler and Scheie syndromes in Japanese populations. *Hum Mutat* 1996; **7**: 23.
44. Scott HS, Bunge S, Gal A *et al*. Molecular genetics of mucopolysaccharidosis type I: diagnostic clinical and biological implications. *Hum Mutat* 1995; **6**: 288.
45. Li P, Wood T, Thompson JN. Diversity of mutations and distribution of single nucleotide polymorphic alleles in the human α -L-iduronidase (IDUA) gene. *Genet Med* 2002; **4**: 420.
46. Chamoles NA, Blanco M, Gaggioli D. Diagnosis of alpha-L-iduronidase deficiency in dried blood spots on filter paper: the possibility of newborn diagnosis. *Clin Chem* 2001; **47**: 780.
47. Gelb MH, Turecek F, Scott CR, Chamoles NA. Direct multiplex assay of enzymes in dried blood spots by tandem mass spectrometry for the newborn screening of lysosomal storage disorders. *J Inherit Metab Dis* 2006; **29**: 397.
48. Shull RM, Kakkis ED, McEntee MF *et al*. Enzyme replacement in a canine model of Hurler syndrome. *Proc Nat Acad Sci USA* 1994; **91**: 12937.
49. Kakkis ED, Muenzer J, Tiller GE *et al*. Enzyme-replacement therapy in mucopolysaccharidosis I. *N Engl J Med* 2001; **344**: 182.
50. Muenzer J, Clark LA, Kolodny EH *et al*. Enzyme replacement therapy for MPS I: 36-week interim results of the phase 3 open-label extension study. Proc Annual Clin Genetic Meeting (ACMG). *Genet Med* 2003; **34**.
51. Dickson PI, Chen AH. Intrathecal enzyme replacement therapy for mucopolysaccharidosis I: translating success in animal models to patients. *Curr Pharm Biotechnol* 2011; **12**: 946-55.
52. Krivit W, Peters C, Shapiro EG. Bone marrow transplantation as effective treatment of central nervous system disease in globoid cell leukodystrophy, metachromatic leukodystrophy, adrenoleukodystrophy, mannosidosis, fucosidosis, aspartylglucosaminuria, Hurler, Maroteaux-Lamy and Sly syndromes, and Gaucher disease type III. *Curr Opin Neurol* 1999; **12**: 167.
53. Whitley CB, Belani KG, Chang PN *et al*. Long term outcome of Hurler syndrome following bone marrow transplantation. *Am J Hum Genet* 1993; **46**: 209.
54. Takahashi Y, Sukegawa K, Aoki M *et al*. Evaluation of accumulated mucopolysaccharides in the brain of patients with mucopolysaccharidoses by H-magnetic resonance spectroscopy before and after bone marrow transplantation. *Pediatr Res* 2001; **49**: 349.

Scheie and Hurler–Scheie diseases/ mucopolysaccharidosis IS and IHS/ α -iduronidase deficiency

Introduction	566	Treatment	570
Clinical abnormalities	567	References	570
Genetics and pathogenesis	569		

MAJOR PHENOTYPIC EXPRESSION

Scheie: stiffness of joints, corneal clouding, disease of the aortic valve, dystosis multiplex, normal intelligence. Hurler–Scheie: intermediate between Hurler and Scheie.

INTRODUCTION

In 1962, Scheie, Hamprick, and Barness [1] described the phenotype as a ‘forme fruste of Hurler’s disease’. This was prescient, as it turned out that the phenotypes are allelic, both resulting from deficiency of the enzyme α -iduronidase (see Figure 77.1). It was the delineation of corrective factors by Fratantoni, Hall, and Neufeld [2] that led to the clear recognition that the Hurler and Scheie genes were allelic, because sulfate accumulation in Hurler fibroblasts was not cross-corrected by Scheie cells, and vice versa [3], and Hurler–Scheie corrective factor was identified as α -L-iduronidase [4]. Both Hurler and Scheie fibroblasts contained no demonstrable α -iduronidase activity against substrate phenyl α -L-iduronide [5, 6].

The intermediate Hurler–Scheie phenotype was first named on clinical grounds by McKusick [7], who postulated that Hurler IH and Scheie IH phenotypes represented homozygosity for one or the other allele and predicted that there would be compounds which expressed an intermediate phenotype that he called IH-S [7]. Actually, it turns out that some of the intermediate phenotypes represent homozygosity for some specific mutations [8]. The cloning of the iduronidase gene on chromosome 4 [9] made it clear that there are many mutations and



Figure 77.1 A seven-year-old boy with Scheie disease illustrates the early claw hand deformities and genu valgum. (Illustration was kindly provided by Dr Philip Benson.)

more to be discovered. Compounds are found even within each of the phenotypes, as are homozygotes. Among the latter with the H-S phenotype are P533R, which is the most common, and particularly common in Morocco [10] where it is the only mucopolysaccharidosis I (MPSI) mutation found to date, and A327P found in Italy and Brazil [11, 12]. The Scheie phenotype was found in Brazil [11] and commonly in Japan [13] with homozygosity for R89Q.

CLINICAL ABNORMALITIES

Scheie disease

The Scheie phenotype has been of particular interest to ophthalmologists, because patients live long enough for the severe corneal clouding to affect vision. It is most dense on the periphery. The patient may first be aware early in the second decade, but it is diagnosable by slit lamp very early. There may also be pigmentary degeneration of the retina. Some patients develop glaucoma. Visual impairment may progress to blindness.

Abnormalities of the joints may be evident early in childhood (Figure 77.1), at least by the age of five years. Joints are stiff and angulated [14]. The claw hand may be identical to that of Hurler disease. Genu valgum is present early. There may be pes cavus and a stiff painful foot. Carpal tunnel syndrome is a common complication due to entrapment of the median nerve [15]. Distal interphalangeal acute angulation gives a trigger-finger appearance [15]. Degenerative arthritis of the hip has been reported [16] along with large femoral cysts and pathologic fracture, but this appears to be rare. Stature is normal.

Facial features may be somewhat coarse, but are often not recognizable as those of a mucopolysaccharidosis. Hypertrichosis is common, and so are inguinal hernias. Some patients develop deafness, and it can be progressive.

Life expectancy may be normal except in those that develop cardiac disease [17]. Aortic stenosis or regurgitation may be evident even early, but, as deposits of mucopolysaccharide increase on the valves and chordae tendinae, disability may develop [18–21]. Sleep apnea was reported in two brothers, 18 and 35 years of age, which was relieved by tracheostomy [22].

Neurologic manifestations are uncommon, but myelopathy has been reported as a consequence of cervical cord compression from thickened dura, the so-called pachymeningitis cervicalis [23, 24]. This problem is more common in the H-S variants.

Hurler–Scheie disease

The clinical features of these patients are intermediate between those of the Hurler and Scheie phenotypes (Figures 77.2, 77.3, 77.4, 77.5, 77.6, 77.7, 77.8, 77.9, 77.10,



Figure 77.2 CL: A 13-year-old girl with Hurler–Scheie disease. Her face in repose showed clear evidence of mucopolysaccharide storage especially about the lips and nose. Corneas were slightly cloudy.



Figure 77.3 CL: She had the claw hand with considerable limitation of motion. There were also contractures at the shoulders and elbows, and she could not raise her hands above her head.

and 77.11). Features may be coarse (Figure 77.2) or not, especially with time (Figure 77.11); an adult patient may have really grotesque features, having lived so much longer than a Hurler patient, and consequently had time to accumulate large amounts of mucopolysaccharide. Some patients have micrognathism, and this may contribute to a distinctive facial appearance [25]. Intellectual functions may be normal; some have impaired mental development. Survival to adulthood is common. Pregnancy has been reported [26]. Stature is short.

Clouding of the cornea is a regular feature of this disorder. In fact, only one patient has been reported with iduronidase deficiency in whom the corneas remained clear (to 14 years at the time of report) [27].



Figure 77.4 HY: A 16-year-old Saudi boy with α -iduronidase deficiency and the clinical phenotype of the Hurler–Scheie syndrome. Formal psychometric testing revealed the intelligence to be normal. He was short; height was 129 cm, and he had coarse facial features, including a large nose and thick lips and hirsutism.



Figure 77.5 HY: There was some micrognathism.



Figure 77.6 HY: There was bilateral clouding of the cornea that ultimately led to corneal transplantation.

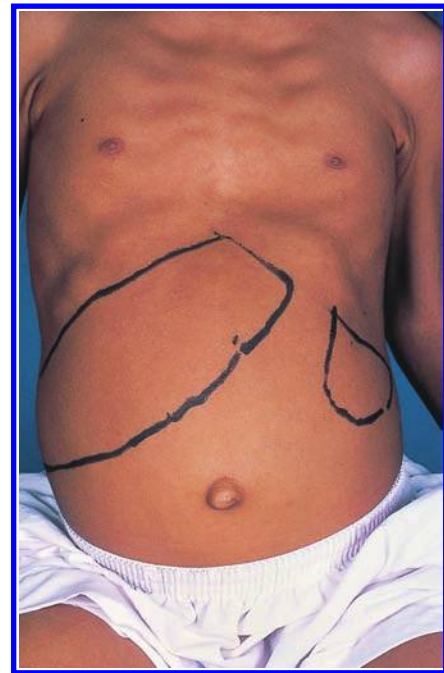


Figure 77.7 HY: Liver and spleen were enlarged as outlined.

Hernias, stiffness of the joints, and the classic claw hand (Figure 77.8) are seen uniformly; so is hepatosplenomegaly (Figure 77.7). Lesions of the cardiac valves may cause cardiac failure and death. Myelopathy from cord

compression is a frequent complication in this condition, as is hydrocephalus resulting from mucopolysaccharide deposition in the meninges [28]. Increased intracranial pressure led to muscle weakness and spasticity attributed to obstruction of the basilar cisterns [28]. This patient presented first at 25 years of age with paranoia. Psychosis has also been observed in the Scheie syndrome. Another patient had marked destruction of the sella and the cribriform plate and spinal fluid rhinorrhea, as well as blindness from pressure on the optic nerves.



Figure 77.8 HY: The hands were broad, short, and flexed, and had a Hurler appearance. There was limitation of joint motion, which was progressive, and he had chronic joint pains.



Figure 77.9 HY: The feet were also short and broad.

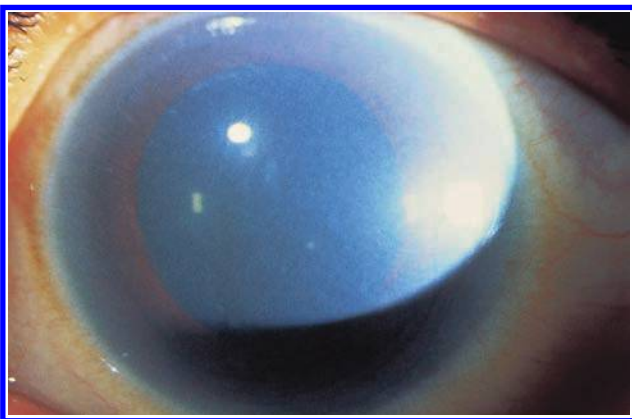


Figure 77.10 AMA: The cornea was quite cloudy and he had glaucoma. Two sisters had Hurler–Scheie disease and all had deficient activity of α -iduronidase. Parents were consanguineous.



Figure 77.11 A 22-year-old with Hurler–Scheie syndrome. Facial features were classic. The enormous tongue had led to respiratory obstruction and tracheotomy. The patient was in intensive care. Liver and spleen were greatly enlarged and he had typical trident hand.

The pathologic appearance of the Hurler–Scheie and of the Scheie disease is that of widespread deposition of mucopolysaccharide [25, 28]. The thickened dura may contain foamy macrophages and increased quantities of collagen. In MPS IS, cortical neurons have been reported as normal, while somatic cells are no different than those in MPS IH. In MPS IHS, changes in cortical neurons are less frequent than those seen in the anterior horn cells of the cord, in which there are typical concentric lamellar inclusions.

GENETICS AND PATHOGENESIS

The Scheie and Hurler–Scheie phenotypes are inherited in autosomal recessive fashion. The fundamental defect is in the α -L-iduronidase [5]. In general, it has not been possible to distinguish the Scheie from more severely affected individuals on the basis of residual enzyme in the usual assays with artificial substrates [29, 30]. Some residual activity has been reported in a radioactive disaccharide assay [31], and others have found some activity in fibroblasts of Scheie patients when desulfated heparan was the substrate [32]. The Scheie disease has been estimated to occur at a frequency of one to 500,000 births in British Columbia [33].

It became clear that patients with the Hurler–Scheie

phenotype have resulted from consanguineous matings [34, 35], indicating that the phenotype may result from homozygosity for single mutant alleles rather than compounds of Hurler and Scheie alleles. Cell hybridization studies of all three phenotypes have led to failure of complementation [36]. These issues have been clarified by the definition of mutations. One patient with the Scheie phenotype has been found to have an allele with a G-to-A transition in intron 5 which creates a new acceptor splice site without losing the original site; thus some normal enzyme is produced [37, 38], as demonstrated by enzyme assay of fibroblasts of the patient [39]. Compound heterozygosity for R89Q, which causes a mild phenotype when homozygous, and 704ins5, which causes a severe phenotype, produced an intermediate pattern of disease in Japanese patients.

TREATMENT

The supportive management and enzyme replacement set out in [Chapter 76](#) is particularly appropriate for Scheie and Hurler–Scheie patients. Corneal transplantation has been successful [40, 41]. Aggressive surgical treatment of glaucoma and carpal tunnel syndrome is also indicated. Cardiac valve replacement has also been successful in both IS and IH/IS patients [18, 19]. Hydrocephalus requires shunting, and cervical cord decompression may be required.

REFERENCES

- Scheie HG, Hamprick GM Jr, Barness LH. A newly recognized form fruste of Hurler's disease (gargoylism). *Am J Ophthalmol* 1962; **53**: 753.
- Fratantoni JC, Hall CW, Neufeld EF. The defect in Hurler's and Hunter's syndromes: faulty degradation of mucopolysaccharides. *Proc Natl Acad Sci USA* 1988; **60**: 699.
- Weismann U, Neufeld EF. Scheie and Hurler syndromes: apparent identity of the biochemical defect. *Science* 1970; **169**: 72.
- Neufeld EF, Cantz MJ. Corrective factors for inborn errors of mucopolysaccharide metabolism. *Ann NY Acad Sci* 1971; **179**: 580.
- Bach G, Friedman R, Weismann B, Neufeld EF. The defect in the Hurler and Scheie syndromes: deficiency of α -L-iduronidase. *Proc Natl Acad Sci USA* 1972; **69**: 2048.
- Matalon R, Dorfman A. Hurler's syndrome and α -L-iduronidase deficiency. *Biochem Biophys Res Commun* 1972; **47**: 959.
- McKusick VA, Howell RR, Hussels IE *et al*. Allelism nonallelism and genetic compounds among the mucopolysaccharidoses. *Lancet* 1972; **1**: 993.
- Li P, Wood T, Thompson JN. Diversity of mutations and distribution of single nucleotide polymorphic alleles in the human α -L-iduronidase (IDUA) gene. *Genet Med* 2002; **4**: 420.
- Schuchman EH, Astrin KH, Aula P, Desnick RJ. Regional assignment of the structural gene for α -L-iduronidase. *Proc Natl Acad Sci USA* 1984; **81**: 1169.
- Alif N, Hess K, Straczek J *et al*. Mucopolysaccharidosis type I: characterization of a common mutation that causes Hurler syndrome in Moroccan subjects. *Ann Hum Genet* 1999; **63**: 9.
- Gatti R, DiNatale P, Villani GR *et al*. Mutations among Italian mucopolysaccharidosis type I patients. *J Inher Metab Dis* 1997; **20**: 803.
- Matte U, Leistner S, Lima L *et al*. Unique frequency of known mutations in Brazilian MPS I patients. *Am J Med Genet* 2000; **90**: 108.
- Yamagishi A, Tomatsu S, Fukuda S *et al*. Mucopolysaccharidosis type I: identification of common mutations that cause Hurler and Scheie syndrome in Japanese populations. *Hum Mutat* 1996; **7**: 23.
- Hamilton E, Pitt P. Articular manifestations of Scheie's syndrome. *Ann Rheum Dis* 1992; **51**: 542.
- MacDougall B, Weeks PM, Wray RC. Median nerve compression and trigger finger in the mucopolysaccharidoses and related diseases. *Plast Reconstr Surg* 1977; **59**: 260.
- Lamon JM, Trojak JE, Abbott MH. Bone cysts in mucopolysaccharidosis I S (Scheie syndrome). *Johns Hopkins Med J* 1980; **146**: 73.
- Dekaban AS, Constantopoulos G, Herman MM, Steusing JK. Mucopolysaccharidosis type V (Scheie syndrome). A postmortem study by multidisciplinary techniques with emphasis on the brain. *Arch Pathol Lab Med* 1976; **100**: 237.
- Pyeritz RE. Cardiovascular manifestations of heritable disorders of connective tissue. In: Steinberg AG, Bearn AG, Motulsky AG, Childs B (eds). *Progress in Medical Genetics*. Philadelphia, PA: Saunders, 1983: 191.
- Butman SM, Karl L, Copelands JG. Combined aortic and mitral valve replacement in an adult with Scheie's disease. *Chest* 1989; **96**: 209.
- Horton WA, Schimke RN. A new mucopolysaccharidosis. *J Pediatr* 1970; **77**: 252.
- Emerit I, Maroteaux P, Vernant P. Deux observations de mucopolysaccharidose avec atteinte cardio-vasculaire. *Arch Franc Pediatr* 1966; **23**: 1075.
- Perks WH, Cooper RA, Bradbury S *et al*. Sleep apnoea in Scheie's syndrome. *Thorax* 1980; **35**: 85.
- Kennedy P, Swash M, Dean MD. Cervical cord compression in mucopolysaccharidosis. *Dev Med Child Neurol* 1973; **15**: 194.
- Paulson GW, Meagler JN, Burkhart J. Spinal pachymeningitis secondary to mucopolysaccharidosis: case report. *J Neurosurg* 1974; **41**: 618.
- Kajii T, Matsuda I, Oshaw AT *et al*. Hurler–Scheie genetic compound (mucopolysaccharidosis IH–IS) in Japanese brothers. *Clin Genet* 1974; **6**: 394.
- Thompson JN, Finley SC, Lorincz AE, Finley WH. Absence of α -L-iduronidase activity in various tissues from two sibs affected with presumably the Hurler–Scheie syndrome. In: Bergsma D (ed.). *Disorders of Connective Tissue*. New York: National Foundation, March of Dimes, 1975: Vol XI, 341.
- Gardner RJM, Hay HR. Hurler's syndrome with clear corneas. *Lancet* 1974; **2**: 845.

28. Winters PR, Harrod MJ, Molenich-Heetred SA *et al.* α -L-iduronidase deficiency and possible Hurler–Scheie genetic compound: clinical pathologic and biochemical findings. *Neurology* 1976; **26**: 1003.
29. Hall CW, Liebaers I, DiNatale P, Neufeld EF. Enzymic diagnosis of the genetic mucopolysaccharide storage disorders. *Meth Enzymol* 1978; **50**: 539.
30. Dinatale P, Leder JG, Neufeld EF. A radio-active substrate and assay for α -L-iduronidase. *Clin Chim Acta* 1977; **77**: 211.
31. Hopwood JJ, Muller V. Biochemical discrimination of Hurler and Scheie syndromes. *Clin Sci* 1979; **57**: 265.
32. Matalon R, Deanching M. The enzymic basis for the phenotypic variation of Hurler and Scheie syndromes. *Pediatr Res* 1977; **11**: 519.
33. Lowry RB, Renwick DHG. The relative frequency of the Hurler and Hunter syndromes. *N Engl J Med* 1971; **284**: 221 (letter).
34. Jensen OA, Pedersen C, Schwartz M *et al.* Hurler–Scheie phenotype: report of an inbred sibship with tapeto-retinal degeneration and electron-microscopic examination of the conjunctiva. *Ophthalmologica* 1978; **176**: 194.
35. Kaibara H, Eguchi M, Shibata K, Takagishi K. Hurler–Scheie phenotype: a report of two pairs of inbred sibs. *Hum Genet* 1979; **53**: 37.
36. Bach G, Moskowitz SM, Tieu PT, Neufeld EM. Molecular analysis of Hurler in Druze and Muslim Arab patients in Israel: multiple allelic mutations of the IDUA gene in a small geographic area. *Am J Hum Genet* 1993; **53**: 330.
37. Scott HS, Litjens T, Nelson PV *et al.* Identification of mutations in the α -L-iduronidase gene (IDUA) that cause Hurler and Scheie syndromes. *Am J Hum Genet* 1993; **53**: 973.
38. Moskowitz SM, Tieu PT, Neufeld EF. Mutation in Scheie syndrome (MPS IS): a GtoA transition creates a new splice site in intron 5 of one IDUA allele. *Hum Mutat* 1993; **2**: 41.
39. Fortuin JJH, Kleijer WJ. Hybridization studies of fibroblasts from Hurler, Scheie and Hurler–Scheie compound patients: support for the hypothesis of allelic mutants. *Hum Genet* 1980; **53**: 155.
40. Wraith JE, Alani SM. Carpal tunnel syndrome in the mucopolysaccharidoses and related disorders. *Arch Dis Child* 1990; **65**: 962.
41. Pronicka E, Tylki-Szymanska A, Kwast O *et al.* Carpal tunnel syndrome in children with mucopolysaccharidoses: needs for surgical tendons and median nerve release. *J Ment Defic Res* 1988; **32**: 79.

Hunter disease/mucopolysaccharidosis type II/ iduronate sulfatase deficiency

Introduction	572	Treatment	577
Clinical abnormalities	572	References	577
Genetics and pathogenesis	576		

MAJOR PHENOTYPIC EXPRESSION

Coarse features, stiff joints, short stature, impaired mental development, hepatosplenomegaly, cardiomegaly, nodular or thickened skin lesions especially over the scapular area, dysostosis multiplex, accumulation of dermatan sulfate and heparan sulfate, and defective activity of iduronate sulfatase.

INTRODUCTION

In 1917, Hunter [1] described two brothers with what is now known as mucopolysaccharidosis (MPS) type II. Patients with the Hunter disease have clinical features similar to those of Hurler disease, although usually they are less severely affected. Patients have been classified clinically into mild and severe forms, although the two cannot be distinguished on the basis of enzyme activity. The advent of molecular analysis and extensive heterogeneity may make

this classification obsolete. Patients with this disease were found, by Dorfman and Matalon [2] and by Muir [3], to excrete dermatan sulfate and heparan sulfate just like those with Hurler disease. It was in studies of Hunter and Hurler cells that Fratantoni, Hall and Neufeld [4] first found the correction factors from each that corrected the defective excess accumulation of sulfate in the other; thus Hurler cells could correct Hunter cells and vice versa, and the Hunter corrective factor would correct Hurler and other MPS cells, but not Hunter cells [5]. The Hunter factor was identified as iduronate sulfatase [6] (Figure 78.1), the enzyme that catalyzes the release of sulfate from the iduronate sulfate moieties of dermatan and heparan sulfates. This is the site of the molecular defect in Hunter disease. Thus, the defect in MPS II is in the first step in the enzymatic breakdown of these mucopolysaccharides.

The gene for iduronate sulfatase has been identified [7] and mapped to the X chromosome at position q28 [8–10]. A number of gross alterations in the gene have been found [7, 11], as well as point mutations, especially at CpG dinucleotides [12, 13].

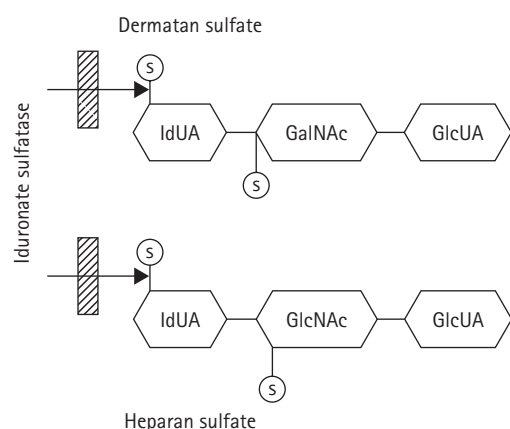


Figure 78.1 Iduronate sulfatase, the site of the enzyme defect in Hunter disease. Both dermatan sulfate and heparan sulfate accumulate when activity is defective.

CLINICAL ABNORMALITIES

Patients with Hunter disease present a broad spectrum of clinical activity; all of them have quite similar reduction in enzyme activity. Nevertheless, the disease has generally



Figure 78.2 CT: A patient with Hunter syndrome. In this most severe form of the disease, the features are quite coarse, the lips very thick, hirsutism prominent, and the hands claw-like, as in Hurler disease. The corneas were clear.

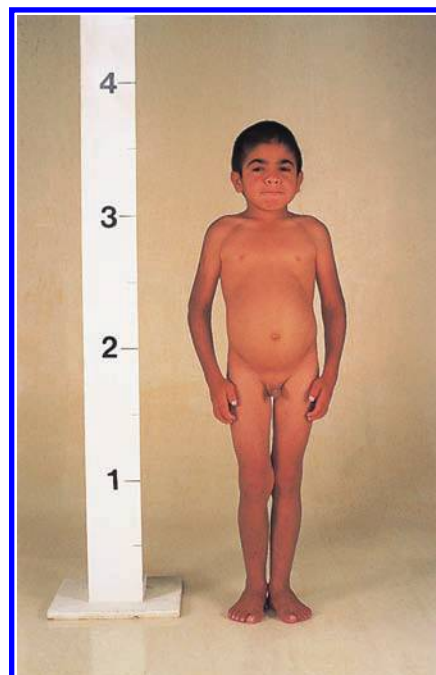


Figure 78.4 AMAQ: A six-year-old boy with a milder expression form of Hunter disease. The hairline was low and the eyebrows bushy. The increased subcutaneous tissue was clearly evident in the anteverted nose, but features were less coarse than the patient in [Figure 78.2](#). Limitation of joint motion was visible in the flexed elbows.



Figure 78.3 ADFS: An eight-year-old boy with Hunter disease. The facial features were quite coarse, the hairline low, and the eyebrows abundant, and the lips were very full. Iduronate sulfatase activity was absent.



Figure 78.5 ADFS: The nodular or pebbly skin lesions constitute a cutaneous marker for the Hurler disease.

been subdivided into two groups, of severe and mild phenotypes, respectively [14–16].

Patients with the severe form may appear identical to those with Hurler disease ([Figures 78.2 and 78.3](#)) except for the absence of cloudy corneas, but behavior is usually quite different. Progression may be slower than in Hurler disease and the apparent onset may be later, often about two to four years of age. In the mild form ([Figure 78.4](#)), mental development may be normal, and lifespan may be long as in Scheie disease.

Hunter disease is distinguished from all other mucopolysaccharidoses by the presence of nodular or pebbly skin lesions ([Figure 78.5](#)), most characteristically over the scapular area, the upper arms or the lateral aspects of the thighs. The skin lesions are sometimes ivory in color. These lesions are not seen in any mucopolysaccharidosis except Hunter disease [17].

As in other forms of mucopolysaccharidosis, chronic respiratory symptoms, rhinorrhea or stertorous breathing, or frequent upper respiratory infections and otitis media



Figure 78.6 AMPAQ: The claw hands were typical for mucopolysaccharidosis.



Figure 78.7 The hand of ADFS was also typical.

may be the earliest manifestations of disease. Presentation for hernia repair may be even earlier. Both inguinal and umbilical hernias are common. Mental development usually continues until at least two years of age.

Patients with the severe form of Hunter disease have the characteristic coarse features of mucopolysaccharidosis (Figures 78.2, 78.3, 78.6, and 78.7) [18]. The nose is flat, the nasal bridge depressed. The lips are thickened, the gums hypertrophic, and the tongue is large. Patients are generally hirsute and have low hairlines (Figures 78.2 and 78.3). The superciliary ridges become very prominent. The head may appear disproportionately large. Stature is short, but this may not be as pronounced as in Hurler disease. Joints are stiff and mobility may become limited, or there may be contractures. Hearing loss is

common; it may not be severe [19], but it tends to be progressive. The hands are broad and the fingers stubby. The claw hand appearance (Figures 78.6 and 78.7) may be indistinguishable from that of Hurler patients. Patients tend to develop high coloration. The liver and spleen are large and hard.

The important negative finding in the Hunter syndrome – the absence of a cloudy cornea [20] – distinguish it from Hurler syndrome. These patients may develop a rounded kyphosis and occasionally there is a severe kyphosis [21]. Corneal clouding may even be detected very late in the most severe forms of Hunter syndrome, but usually only with a slit lamp [22].

The voice is hoarse. Diarrhea may be a chronic problem; it may result from infiltration of the autonomic innervation



Figure 78.8 AMPAQ: Roentgenograms of the hands illustrate the broadened phalanges and metacarpals, as well as the fixed flexion deformities. The proximal ends of the metacarpals are tapered.

of the intestine [23]. Retinitis pigmentosa may occur in this condition and retinal degeneration may cause blindness. Glaucoma may be a problem. Papilledema may be seen [24]; this is probably a consequence of pachymeningeal thickening, which may also lead to neurologic defects including quadriplegia from pressure on the cord [25]. It may also result in hydrocephalus [26]. Cerebral atrophy, which may also lead to ventricular enlargement, is seen regularly on computed tomography (CT) scan or magnetic resonance imaging (MRI) in severe Hunter disease [27–29], and there may be defective reabsorption of cerebrospinal fluid. Intracranial pressure may be increased.

Mental deterioration is progressive, but usually occurs at a slower rate than in Hurler disease. Rarely mental deterioration may be profound early in life.

The behavior of a patient with severe Hunter disease is often characteristic [19] and contrasts sharply with the sweet disposition of the Hurler patient. From two to six years of age, the Hunter patient may develop primitive, uncontrolled activity in which he throws toys and seems to enjoy self-created noise. He is hyperkinetic. Rough, aggressive play may be dangerous to pets or younger siblings. These patients often are stubborn, fearless, and unresponsive to discipline. Eating habits may be unusual, and pica is common. One patient developed lead poisoning. The management of such a child is difficult and admission to an institution is common.

Obstructive airway disease may result from infiltration of the vocal cords or trachea, or a large tongue. Tracheostomy may be necessary. Cardiac complications, such as congestive failure, result from valvular or myocardial infiltration. Coronary insufficiency may result from infiltration in the vessels. Thickened valves may be demonstrated by echocardiography. Some have pulmonary hypertension.

Most patients deteriorate progressively after five or six years of age. Physical activity decreases; gait may become unsteady; and speech deteriorates and ultimately is lost. Difficulty in ingesting solid food is progressive, and there is loss of weight. Respiratory infections become more frequent and more severe and may be the cause of death. Generalized seizures may occur in the final months of life. Death usually occurs by 15 years of age from respiratory or cardiac disease.

Patients with the milder forms of the disease may survive well into their sixties or beyond [30–33]. Intelligence may be preserved. Features may appear normal in childhood, or may be mildly coarse (Figure 78.4), but with time the appearance becomes increasingly recognizable. Joint stiffness may be an increasing problem, and patients may develop osteoarthritis. Carpal tunnel syndrome is common.

Hearing loss is regularly observed. Retinal dysfunction may be documented by electroretinography [34]. Chronic papilledema has been reported in the absence of increased intracranial pressure [35, 36]. Hydrocephalus appears to be rare in the mild forms of Hunter disease [28]. Arachnoid cysts have been observed [37]. Spinal stenosis, especially cervical may cause cord compression [38].

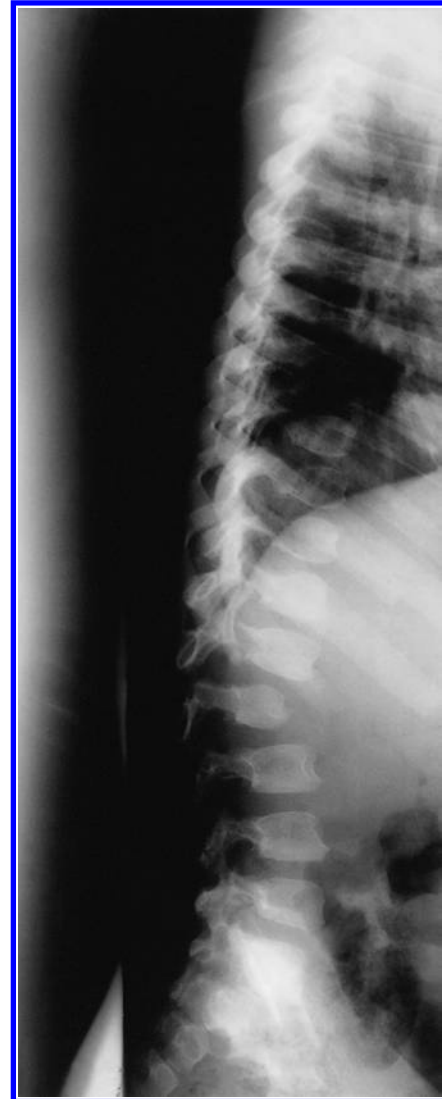


Figure 78.9 AMAQ: The L2 vertebra was beaked and displaced posteriorly.



Figure 78.10 The skull of AMAQ illustrates the thickened cranial vault.

Survival as long as 87 years has been observed [31], but death may occur in the second decade, even in the mild phenotypes. The cause may be cardiac disease, pulmonary infection, or airway obstruction.

The roentgenographic picture of all forms of the disease is that of dysostosis multiplex. The features may be quite similar to those of the Hurler disease, but they tend to be less dramatic (Figures 78.8, 78.9, and 78.10). External thickening of the cortices of the bones may be seen early. With increasing age the cortical walls become thinner as the marrow cavities expand [39]. The skull is large and the sella shoe-shaped. The lower ribs are broad and spatulate. There is hypoplasia of the vertebrae and beaking of L2 (Figure 78.9). Large radiolucent areas surrounding the unerupted teeth represent dentigenous cysts, not accumulation of mucopolysaccharide [40]. Smaller lucent lesions may be collections of collagen.

Fundamental to the clinical phenotype is the excessive intracellular accumulation of acid mucopolysaccharides. Large vacuolated cells containing metachromatic cytoplasmic material are present on histologic examination of many tissues. In the scapular nodules there is extracellular accumulation of metachromatic material [41]. Dermatan sulfate and heparan sulfate [2, 14] are excreted in the urine in large and approximately equal amounts. Cultured fibroblasts show metachromatic staining and contain large amounts of mucopolysaccharide [2]. Hunter cells accumulate labeled sulfate in a typical mucopolysaccharidosis pattern.

GENETICS AND PATHOGENESIS

The Hunter disease is inherited as an X-linked recessive trait. Patients with specific mild or severe phenotypes closely resemble other affected members of an individual family. The incidence of the disease has approximated one in 100,000 male births in Great Britain and British Columbia [42–44], and one in 36,000 in Israel [45, 46]. The disease has been recognized in a small number of female individuals. One had an X:5 autosome translocation in which the breakpoint at the gene locus caused the disease, because the normal X was inactivated [47, 48]. Others represented nonrandom inactivation of a normal X chromosome, including one of a pair of nonidentical twins [49–51].

The molecular defect is in the enzyme iduronate sulfatase (Figure 78.1) [6, 52, 53]. The enzyme has been purified from human liver [54], placenta [55], and plasma [56]. The human cDNA codes for a polypeptide of 550 amino acids [10]. The enzyme [57], which removes the sulfate from the 2-position of iduronic acid, is essential for the sequential degradation of heparan sulfate, which contains many sulfated iduronic acid residues, and dermatan sulfate, which contains a smaller number of such residues. The failure to degrade even a single sulfated uronic acid leads to the accumulation of the glycosaminoglycan. Enzymatic

analysis for the activity of iduronate sulfatase fails to distinguish among the mild and severe clinical phenotypes; in all of them there is virtually complete absence of enzyme activity.

β -Galactosidase activity is diminished in the skin and other tissues of patients with the disease [58]. This abnormality, which also occurs in Hurler disease, is secondary to the primary defect, but it could relate to the accumulation of ganglioside and other lipids found in the brain of patients.

The diagnosis, once suspected clinically, has been confirmed by the quantitative assay of the excretion of total glycosaminoglycans in the urine, but the specific diagnosis depends on the assay for iduronate sulfatase, which can be carried out on serum, cells, or tissues [59]. None of the screening tests for mucopolysaccharide in the urine is completely reliable.

Heterozygous female carriers of the Hunter gene have been recognized by cloning of fibroblasts followed by assessment of the accumulation of ^3S -mucopolysaccharide [60] or assay of iduronate sulfatase in individual hair roots [61, 62]. Two clonal populations, one normal and the other abnormal, have been demonstrated using both of these techniques, as specified by the Lyon hypothesis. Demonstration of a major deletion in the gene provides a highly accurate and less demanding approach to carrier detection.

Prenatal diagnosis was initially carried out successfully by using sulfate incorporation in cultured amniocytes [63]. It is now done by assay of the enzyme in amniocytes or in amniotic fluid. It is recommended that the early information obtained from the fluid always be confirmed by assay of the cultured cells. Prenatal diagnosis has also been accomplished by assay of the enzyme in chorionic villus homogenates [64]. In the case of female fetuses, very low levels of enzyme may be found in either amniocytes or chorionic villus cells; therefore, it is important that karyotyping be carried out in all instances. In families in which the mutation is known, molecular methods are of choice for prenatal diagnosis and heterozygote detection.

The gene for iduronate sulfatase is very large. It contains nine exons over 24 kb [65, 66]. Complete or partial deletions of the gene were identified in patients with the severe phenotype [57, 67]. Among this population of patients with severe disease, deletions or rearrangements visible in Southern blots occur in about 20 percent [7, 11, 68–70]. It appears that this area is structurally susceptible to major alterations, because identical changes have been found in unrelated patients [66]. In addition, a number of missense and nonsense mutations have been identified [11–13, 67, 71–75]. Approximately half of the single base substitutions have occurred at CpG dinucleotides, suggesting independent origin in different families [76]. Codon R468 when changed to W led to mild disease in a patient in the United States [13] and severe disease in a Japanese [70], typifying the problem of genotype–phenotype correlation; it was also changed to Q, L, and G

in severely affected patients [13, 77, 78]. New mutation has been found to occur more frequently in the genesis of the heterozygous carrier than of the affected male [70, 76].

TREATMENT

Specific treatment for this disease continues to be explored. Enzyme replacement therapy has not been effective to date. Bone marrow transplantation has been performed in this disease, but most experience is with MPS I (Chapters 76 and 77) [79]. It is not currently recommended in Hunter disease.

A variety of supportive measures are useful, especially in the milder forms of the disease, in which longer survival is associated with some painful complications. Shunting is important in the management of hydrocephalus. Hearing aids may aid in deafness. Physiotherapy is useful for the joint stiffness and the avoidance of contractures. Surgical decompression is carried out for carpal tunnel syndrome. Cardiac valvular status should be monitored by echocardiography. Tracheostomy or nasal continuous positive airway pressure may alleviate obstructive airway disease.

REFERENCES

- Hunter C. A rare disease in two brothers. *Proc R Soc Med* 1917; **10**: 104.
- Dorfman A, Matalon R. The Hurler and Hunter syndromes. *Am J Med* 1969; **47**: 691.
- Muir H. The structure and metabolism of mucopolysaccharidoses. *Am J Med* 1969; **47**: 673.
- Fratantoni JC, Hall CW, Neufeld EF. Hurler and Hunter syndromes: mutual correction of the defect in cultured fibroblasts. *Science* 1968; **162**: 570.
- Cantz M, Chrambach A, Bach G, Neufeld EF. The Hunter corrective factor. *J Biol Chem* 1972; **247**: 5456.
- Bach G, Eisenberg F, Cantz M, Neufeld EF. The defect in the Hunter syndrome: deficiency of sulfiduronate sulfatase. *Proc Natl Acad Sci USA* 1973; **70**: 2134.
- Palmieri G, Capra V, Romano G *et al.* The iduronate sulfatase gene: isolation of a 12 Mb YAC contig spanning the entire gene and identification of heterogeneous deletions in patients with Hunter syndrome. *Genomics* 1992; **12**: 52.
- Upadhyaya M, Sarfarazi M, Bamforth JS *et al.* Localization of the gene for Hunter syndrome on the long arm of X-chromosome. *Hum Genet* 1986; **74**: 39.
- Le Guern E, Couillin P, Oberle I *et al.* More precise localization of the gene for Hunter syndrome. *Genomics* 1990; **7**: 358.
- Wilson PJ, Morris CP, Anson DS *et al.* Hunter syndrome: isolation of an iduronate-2-sulfatase cDNA clone and analysis of patient DNA. *Proc Natl Acad Sci USA* 1990; **87**: 8531.
- Wraith JE, Cooper A, Thornley M *et al.* The clinical phenotype of two patients with a complete deletion of the iduronate-2-sulfatase gene (mucopolysaccharidosis type II Hunter syndrome). *Hum Genet* 1991; **87**: 205.
- Sukegawa K, Tomatsu S, Katsuyuki T *et al.* Intermediate form of mucopolysaccharidosis type II (Hunter disease): a C1327 to T substitution in the iduronate sulfatase gene. *Biochem Biophys Res Commun* 1992; **183**: 809.
- Crotty PL, Braun SE, Anderson RA, Whitley CB. Mutation R468W of the iduronate-2-sulfatase gene in mild Hunter syndrome (mucopolysaccharidosis type II) confirmed by *in vitro* mutagenesis and expression. *Hum Mol Genet* 1992; **1**: 755.
- Spranger J. The systemic mucopolysaccharidoses. *Ergeb Inn Med Kinderheilkd* 1972; **32**: 165.
- Young ID, Harper PS, Archer IM, Newcombe RG. A clinical and genetic study of Hunter's syndrome. 1 Heterogeneity. *J Med Genet* 1982; **19**: 401.
- Young ID, Harper PS, Newcombe RG, Archer IM. A clinical and genetic study of Hunter's syndrome. Two differences between the mild and severe forms. *J Med Genet* 1982; **19**: 408.
- Prystowsky SD, Maumenee IH, Freeman RG *et al.* A cutaneous marker in the Hunter syndrome: a report of four cases. *Arch Dermatol* 1977; **113**: 602.
- Young ID, Harper PS. The natural history of the severe form of Hunter's syndrome: a study based on 52 cases. *Dev Med Child Neurol* 1983; **25**: 481.
- Leroy JG, Crocker AC. Clinical definition of the Hurler-Hunter phenotypes. *Am J Dis Child* 1966; **112**: 518.
- McKusick VA. *Heritable Disorders of Connective Tissue*, 4th edn. St Louis: CV Mosby, 1972: 5556.
- Benson PF, Button LR, Fensom AH, Dean MF. Lumbar kyphosis in Hunter's disease (MPSII). *Clin Genet* 1979; **16**: 317.
- Spranger J, Cantz M, Gehler J *et al.* Mucopolysaccharidosis II (Hunter disease) with corneal opacities: report of two patients at the extremes of a wide clinical spectrum. *Eur J Pediatr* 1978; **129**: 11.
- Elsner B. Ultrastructure of the rectal wall in Hunter's syndrome. *Gastroenterology* 1970; **58**: 856.
- Young ID, Harper PS. Long-term complications in Hunter's syndrome. *Clin Genet* 1978; **16**: 125.
- Ballenger CE, Swift TR, Leshner RT *et al.* Myelopathy in mucopolysaccharidosis type II (Hunter syndrome). *Ann Neurol* 1980; **7**: 382.
- Yatziv S, Epstein CJ. Hunter syndrome presenting as macrocephaly and hydrocephalus. *J Med Genet* 1997; **14**: 445.
- Gibbs DA. Computed tomography studies on patients with mucopolysaccharidoses. *Neuroradiology* 1981; **21**: 9.
- Van Aerde J, Plets C, van der Hauwaert L. Hydrocephalus in Hunter syndrome. *Acta Paediatr Belg* 1981; **34**: 93.
- Timms KM, Bondeson ML, Ansari-Lari MA *et al.* Molecular and phenotypic variation in patients with severe Hunter syndrome. *Hum Mol Genet* 1997; **6**: 479.
- Young ID, Harper PS. Mild form of Hunter's syndrome: clinical delineation based on 31 cases. *Arch Dis Child* 1982; **57**: 828.
- Hobolth N, Pedersen C. Six cases of mild form of the Hunter syndrome in five generations. Three affected males with progeny. *Clin Genet* 1978; **13**: 121.
- Differante NM, Nichols BL Jr. A case of the Hunter syndrome with progeny. *Johns Hopkins Med J* 1978; **130**: 121.

33. Karpati G, Carpenter S, Eisan AA *et al.* Multiple peripheral nerve entrapments: an unusual phenotypic variant of the Hunter syndrome (mucopolysaccharidosis II) in a family. *Arch Neurol* 1974; **31**: 418.
34. Caruso RC, Kaiser-Kupfer MI, Muenzer J *et al.* Electroretinographic findings in the mucopolysaccharidoses. *Ophthalmology* 1986; **93**: 1612.
35. Beck M, Cole G. Disc oedema in association with Hunter's syndrome: ocular histopathological findings. *Br J Ophthalmol* 1984; **68**: 590.
36. Beck M. Papilloedemas in association with Hunter syndrome. *Br J Ophthalmol* 1983; **67**: 174.
37. Neuhauser EBD, Griscom NT, Gilles FH, Crocker AC. Arachnoid cysts in the Hurler–Hunter syndrome. *Ann Radiol* 1968; **11**: 453.
38. Vinchon M, Cotten A, Clarisse J *et al.* Cervical myelopathy secondary to Hunter syndrome in an adult. *Am J Neuroradiol* 1995; **16**: 1402.
39. Caffey J. Gargoylism (Hunter–Hurler disease dysostosis multiplex lipochondrodystrophy). *Am J Roentgenol Radium Ther Nucl Med* 1952; **67**: 715.
40. Lustmann J, Bimstein E, Yatziv S. Dentigerous cysts and radiolucent lesions of the jaw association with Hunter's syndrome. *J Oral Surg* 1975; **33**: 679.
41. Freeman RG. A pathological basis for the cutaneous papules of mucopolysaccharidosis II (the Hunter syndrome). *J Cutan Pathol* 1977; **4**: 673.
42. Young ID, Harper PS. Incidence of Hunter's syndrome. *Hum Genet* 1982; **60**: 391.
43. Lowry RB, Renwick DHG. Relative frequency of the Hurler and Hunter syndromes. *N Engl J Med* 1971; **284**: 221.
44. Lowry RB, Applegarth DA, Toone JR *et al.* An update on the frequency of mucopolysaccharide syndromes in British Columbia. *Hum Genet* 1990; **85**: 389.
45. Zlotogora J, Schaap T, Zeigler M, Bach G. Hunter syndrome in Jews in Israel: further evidence for prenatal selection favoring the Hunter allele. *Hum Genet* 1991; **86**: 531.
46. Chakravarti A, Bale SJ. Differences in the frequency of X-linked deleterious genes in human populations. *Am J Hum Genet* 1983; **35**: 1252.
47. Mossman J, Blunt S, Stephens R *et al.* Hunter's disease in a girl: association with X: 5 chromosomal translocation disrupting the Hunter gene. *Arch Dis Child* 1983; **58**: 911.
48. Roberts SH, Upadhyaya M, Sarfarazi M, Harper PS. Further evidence localizing the gene for Hunter's syndrome to the distal region of the X-chromosome long arm. *J Med Genet* 1989; **26**: 309.
49. Clarke JTR, Greer WL, Strasberg PM *et al.* Hunter disease (mucopolysaccharidosis type II) associated with unbalanced inactivation of the X chromosome in a karyotypically normal girl. *Am J Hum Genet* 1991; **4**: 289.
50. Clarke JTR, Wilson PJ, Morris CP *et al.* Characterization of a deletion at Xq27–28 associated with unbalanced inactivation of the nonmutant X-chromosome. *Am J Hum Genet* 1992; **51**: 316.
51. Winchester B, Young E, Geddes S *et al.* Female twin with Hunter disease due to nonrandom inactivation of the X-chromosome: a consequence of twinning. *Am J Med Genet* 1992; **44**: 834.
52. Sjoberg I, Fransson LA, Matalon R, Dorfman A. Hunter's syndrome: a deficiency of l-idurono-sulfate sulfatase. *Biochem Biophys Res Commun* 1973; **54**: 1125.
53. Liebaers I, Neufeld EF. Iduronate sulfatase activity in serum lymphocytes and fibroblasts – simplified diagnosis of the Hunter syndrome. *Pediatr Res* 1976; **10**: 733.
54. Bielicki J, Freeman C, Clements PR, Hopwood JJ. Human liver iduronate-2-sulphatase: purification characterization and catalytic properties. *Biochem J* 1990; **271**: 75.
55. DiNatale P, Daniele A. Iduronate sulfatase from human placenta. *Biochem Biophys Acta* 1985; **839**: 258.
56. Wasteson A, Neufeld EF. Iduronate sulfatase from human plasma. *Methods Enzymol* 1982; **83**: 573.
57. Bielicki J, Freeman C, Clements PR, Hopwood JJ. Human liver iduronate-2-sulphatase. Purification, characterization and catalytic properties. *Biochem J* 1990; **271**: 75.
58. Gerich JE. Hunter's syndrome Beta-galactosidase deficiency in skin. *N Engl J Med* 1969; **280**: 799.
59. Yatziv S, Erickson RP, Epstein CJ. Mild and severe Hunter syndrome (MPSII) within the sibships. *Clin Genet* 1977; **11**: 319.
60. Migeon BR, Sprenkle JA, Liebaers I *et al.* X-linked Hunter syndrome: the heterozygous phenotype in cell culture. *Am J Hum Genet* 1977; **29**: 448.
61. Yutaka T, Fluharty AL, Stevens RL, Kihara H. Iduronate sulfatase analysis of hair roots for identification of Hunter syndrome heterozygotes. *Am J Hum Genet* 1978; **30**: 575.
62. Nwokoro N, Neufeld EF. Detection of Hunter heterozygotes by enzymatic analysis of hair roots. *Am J Hum Genet* 1979; **31**: 42.
63. Frattantoni JC, Neufeld EF, Uhlendorf W, Jacobson CB. Intrauterine diagnosis of the Hurler and Hunter syndromes. *N Engl J Med* 1969; **280**: 686.
64. Kleijer WJ, Van Diggelen OP, Janse HC *et al.* First trimester diagnosis of Hunter syndrome on chorionic villi. *Lancet* 1984; **2**: 472.
65. Flomen RH, Green EP, Bentley DR, Giannelli F. Determination of the organisation of coding sequences within the iduronate sulphate sulphatase (IDS) gene. *Hum Mol Genet* 1993; **2**: 5.
66. Wilson PJ, Meaney CA, Hopwood JJ, Morris CP. Sequence of the human iduronate 2-sulfatase (IDS) gene. *Genomics* 1993; **17**: 773.
67. Wilson PJ, Suthers GK, Callen DF. Frequent deletions at Xq28 indicate genetic heterogeneity in Hunter syndrome. *Hum Genet* 1991; **86**: 505.
68. Steen-Bondeson ML, Dahl N, Tonnesen T *et al.* Molecular analysis of patients with Hunter syndrome: implication of a region prone to structural alterations within the EDS gene. *Hum Mol Genet* 1992; **1**: 195.
69. Bunge S, Steglich C, Beck M *et al.* Mutation analysis of the iduronate 2-sulfatase gene in patients with mucopolysaccharidosis type II (Hunter syndrome). *Hum Mol Genet* 1992; **1**: 335.
70. Beck M, Steglich C, Zabel B *et al.* Deletion of the Hunter gene and both DXS466 and DXS304 in a patient with mucopolysaccharidosis type II. *Am J Med Genet* 1992; **44**: 100.

71. Bunge S, Steglich C, Beck M *et al.* Mutation spectrum of the iduronate-2-sulfatase gene in patients with Hunter syndrome. *Am J Hum Genet* 1992; **51**: A166.
72. Hopwood JJ, Bunge S, Morris CP *et al.* Molecular basis of mucopolysaccharidosis type II: mutations in the iduronate 2-sulphatase gene. *Hum Mutat* 1993; **2**: 435.
73. Muenzer J, Tütera M. Molecular analysis of iduronate sulfatase mutations in mucopolysaccharidosis II (Hunter syndrome). *Am J Hum Genet* 1992; **51**: A174.
74. Flomen RH, Green PM, Bentley DR *et al.* Detection of point mutations and a gross deletion in six Hunter syndrome patients. *Genomics* 1992; **13**: 543.
75. Froissant R, Maire I, Millat G *et al.* Identification of iduronate sulfatase gene alterations in 70 unrelated Hunter patients. *Clin Genet* 1998; **53**: 362.
76. Rathmann M, Bunge S, Beck M *et al.* Mucopolysaccharidosis type II (Hunter syndrome): mutation 'hot spots' in the iduronate-2-sulfatase gene. *Am J Hum Genet* 1996; **59**: 1202.
77. Isogai K, Sukegawa K, Tomatsu S *et al.* Mutation analysis in the iduronate-2-sulphatase gene in 43 Japanese patients with mucopolysaccharidosis type II (Hunter disease). *J Inher Metab Dis* 1998; **21**: 60.
78. Whitley CB, Anderson RA, Aronovich EL *et al.* Caveat to genotype-phenotype correlation in mucopolysaccharidosis type II: discordant clinical severity of R468W and R468Q mutations of the iduronate-2-sulfatase gene. *Hum Mutat* 1993; **2**: 235.
79. Krivit W, Shapiro E, Hoogerbrugge PM, Moser HW. State of the art review. Bone marrow transplantation treatment for storage diseases. *Bone Marrow Transplant* 1992; **10**: 87.

Sanfilippo disease/mucopolysaccharidosis type III

Introduction	580	Treatment	585
Clinical abnormalities	580	References	586
Genetics and pathogenesis	585		

MAJOR PHENOTYPIC EXPRESSION

Severe mental deterioration, mild skeletal dysostosis multiplex, and urinary excretion of heparan sulfate. The Sanfilippo disease type A is due to a deficiency of heparan-N-sulfatase, type B to a deficiency of α -N-acetylglucosaminidase, type C to acetylCoA: α -glucosaminide acetyl transferase, and type D to N-acetylglucosamine-6-sulfatase.

INTRODUCTION

This disorder was first described by Harris [1] in 1961 with the report of a six-year-old girl with mildly impaired mental development who had hepatosplenomegaly and a normal skeletal survey and excreted large amounts of heparan sulfate in the urine. Sanfilippo and colleagues [2, 3] in 1962 and 1963 described eight children with a wide range in degree of impaired mental development, all of whom had heparan sulfate mucopolysacchariduria. Some of these patients had similarities, in appearance and in roentgenographic findings, to patients with the Hurler and Hunter syndromes. The syndrome is characterized chemically by the exclusive excretion of heparan sulfate in the urine, which distinguishes it from all of the other mucopolysaccharidoses. It is also clinically unique in the disparity between the generally severe cerebral degeneration and the relatively mild effects on the skeleton, viscera, and facial features [4].

Fibroblasts derived from patients with the Sanfilippo disease accumulate $^{35}\text{SO}_4$. The existence of more than one type of disease was first recognized through cross-correction studies [5]. Patients initially studied fell into two groups, and those of each group could correct the other. The correction factors are the enzymes whose activity is lacking in each of the types. In type A Sanfilippo cells, Kresse and Neufeld [6] found that the defective enzyme is heparan-N-sulfatase (Figures 79.1 and 79.2). In type B, the defect was found by O'Brien [7] and by von Figura and Kresse [8] to be in α -N-acetylglucosaminidase (Figure 79.1). The latter

group defined the acetyltransferase defect in type C [9] and the N-acetylglucosamine-6-sulfatase abnormality in type D disease [10]. The cDNA for this IIID disease gene has been cloned [11] and mapped to chromosome 12q14 [12]. The cDNA for the IIIA enzyme was cloned and mapped to chromosome 17q25 [13]. The gene for IIIB was cloned and mapped to chromosome 17q21 [14]. A relatively small number of mutations, predominantly missense and private to an individual family has been found in the IIIA and IIIB genes [15, 16].

CLINICAL ABNORMALITIES

The clinical features of each of the four Sanfilippo disease types are indistinguishable (Figures 79.3, 79.4, 79.5, 79.6, 79.7, 79.8, 79.9, and 79.10). Patients are characteristically normal in appearance at birth and appear to develop normally during the first year. They usually are referred after one or two years of age because of slowness in development or after three or four years because of delayed speech. They may have had difficulty in feeding, especially with solids, as well as repeated respiratory infections from the beginning. Impaired mental development becomes progressively more obvious with time. These patients do not have abnormalities in linear growth, and muscle strength is good. Progressive degeneration occurs to a severe degree of mental incapacitation, although there is variability among patients. Skills learned during the first years are lost, including speech and toilet training. Others never learn to speak, while speech in some is lost well after

the first decade; some patients develop some impairment of hearing. Neurologic problems are progressive. The gait becomes clumsy and coordination poor. Deep tendon reflexes are accentuated. Purposeless athetoid-like movements may develop. The patient may drool constantly. There may be seizures, but anticonvulsant control is not difficult. Finally, the patient becomes bedridden and gastrostomy or nasogastric feeding is required. Death usually supervenes before the 20th birthday or even before the tenth [4], but survival into the third or fourth decade is possible [17].

Management of behavior may be a problem and may even be the presenting complaint. Behavior tends to

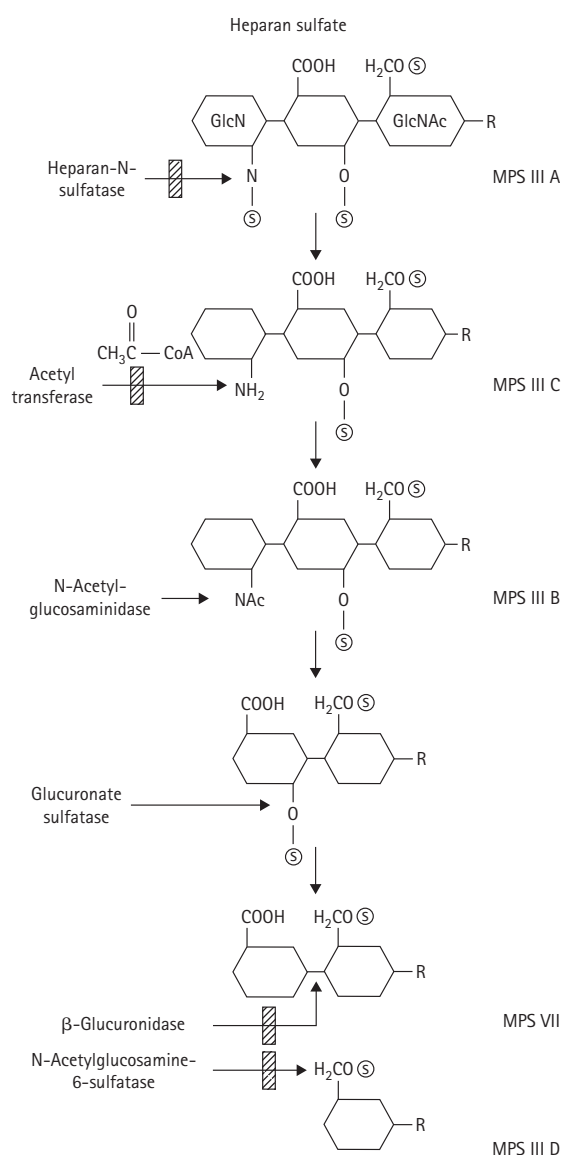


Figure 79.1 The defect in Sanfilippo disease type A is in heparan-N-sulfatase, while in type B it is α -N-acetylglucosaminidase. In type C, it is an acetyl transferase, and in type D, it is N-acetylglucosamine-6-sulfatase. The phenotypes of the various types are indistinguishable.

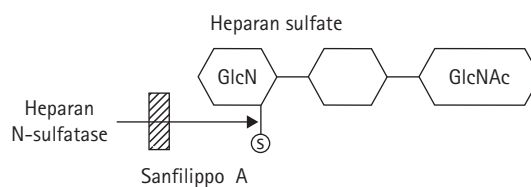


Figure 79.2 Heparan sulfate accumulates in Sanfilippo disease.



Figure 79.3 CF: An 11-year-old girl with Sanfilippo A disease. The thick alae nasi were clear evidence of mucopolysaccharide storage, but features were otherwise not coarse. She had moderate hirsutism and severe hypertonia, as indicated by her hands. Liver was palpable at 4 cm.



Figure 79.4 CL: A 21-year-old female with Sanfilippo A disease. She was thin, dystonic, and wheelchair-bound.



Figure 79.5 RGQ: A 21-month-old girl with Sanfilippo disease type A. She was quite hirsute, and the facial features were somewhat coarse. The liver was palpable at 8 cm. Growth was normal, but she was developmentally delayed.



Figure 79.7 HMZ: A 2½-year-old boy with Sanfilippo disease type B. The diagnosis was made because of a positive family history. Features were not coarse, but he was more hirsute than the unaffected members of the family. Development was slow; he had only a few words, but he had walked at 18 months.



Figure 79.6 GGQ: A nine-year-old girl with Sanfilippo disease, the sister of the patient in Figure 79.5. The history was of loss of developmental milestones, such as walking. She was very hirsute. There was no organomegaly. The calvaria was thickened, but she did not have dystosis multiplex. On follow up by 16 years, she had profoundly impaired mental development and had spasticity, had contractures, and was unresponsive to social stimuli.



Figure 79.8 ARAZ: A four-year-old boy with Sanfilippo disease type B. Mental deficiency increased progressively after two years of age, and facial features coarsened. Hirsutism was prominent. By five years, his cognitive mental age on the Bayley scale was nine months, but motor performance was spared.



Figure 79.9 HRIH: A 12-year-old boy with Sanfilippo disease type B. He was hirsute and his facial features were coarse, especially about the nose and lips; mental deficiency was severe.



Figure 79.10 AS: A six-year-old girl with Sanfilippo disease type D. She had progressive intellectual deterioration. She had no speech and autistic behavior, but facial features were unremarkable. Activity of N-acetylglucosamine-6-sulfatase was 5 percent of control.

become worse as, with age, patients become increasingly stubborn and withdrawn; many are hyperactive. Disorders of sleep and insomnia are common and some are up all night, at least on occasion. Chewing the bedclothes and sudden crying out are common. Inappropriate laughing or singing is less common. Patients may be aggressive, and temper tantrums occur. Patients may have pica and eat unusual objects. Interaction with other children may

be difficult. They can be destructive and dangerous to siblings. The combination of aggressive behavior, profound dementia, and normal physical strength is a daunting one. They often are so difficult to handle that admission to an institution is common [18]. Some patients have come to attention as adults with psychiatric disease, even of a type requiring admission to a closed ward [19]. Drug treatment of the behavior is seldom effective [20].

There may be differences in the severity of disease in the different types. Adult onset of dementia and minimal somatic disease have been reported in type B [21]; while dementia is commonly observed by six years of age in type A [18]. In addition, early-onset progression tends to be more rapid in type A than in types B and C. However, there is considerable heterogeneity. A particularly severe type A disease has been reported [22] from the Cayman Islands. In both types A and B, severe and mild forms of the disease have been reported in the same sibship [23, 24]. Type C severity may be intermediate between that of types A and B or may present in infancy [25]. Type D is rare, but also heterogeneous [26–28].

The features of the patient with Sanfilippo disease usually become somewhat coarse (Figures 79.4, 79.5, 79.6, and 79.7), but often the patient is not recognizable as having a mucopolysaccharidosis. In some the features are not recognizably coarse (Figures 79.9 and 79.10). In our experience, programs of screening of the urine for metabolic disease of unselected patients in institutions for the mentally impaired are more likely to bring to light previously undiagnosed patients with this disorder than any other disease of metabolism.

The bridge of the nose may be slightly flattened and the lips somewhat thick. Many of the patients are hirsute; the eyebrows may be bushy and the hairline low. Some have had macrocephaly. The dull, rigid facies is a consequence of cerebral deterioration, as contrasted with the local tissue changes of Hurler disease. Some patients may have a mild limitation of joint mobility. Hepatosplenomegaly may be mild, especially in childhood; it is more often undetectable in adulthood. There is no gibbus, and the corneas are clear. Cardiac abnormalities have not usually been observed in these patients [17]; however, a patient has been reported [29] in whom there was severe incapacitating involvement of the mitral valve. Hernias may be a problem and may recur after correction. Hearing loss may be progressive and severe. Watery diarrhea may be a recurrent problem in childhood. An early onset of puberty may be observed [30].

Roentgenographic findings are those of a mild dystosis multiplex (Figures 79.11, 79.12, and 79.13) [17]. Most patients with this syndrome have a thickening and increased density of the cranial vault in the posterior parietal and occipital areas (Figure 79.12) [31]. The mastoids may be sclerotic. The sella turcica appears normal. There may be a biconvex appearance or an ovoid dysplasia of the thoracolumbar vertebrae, as well as platyspondyly (Figure 79.11). Among patients with dysostosis multiplex, those with this syndrome have the mildest bony changes. Those



Figure 79.11 Roentgenogram of the spine of a 16-year-old boy with Sanfilippo disease. There was mild platyspondyly, and the ribs were spatulate with posterior narrowing. This is consistent with dysostosis multiplex, but appreciably milder than those of other forms of mucopolysaccharidosis. Roentgenograms of his extremities were normal.



Figure 79.12 Roentgenogram of the skull of the 16-year-old shows increase in thickness of the diploic space, especially posteriorly. This is characteristic of this disease. The sella turcica is normal.

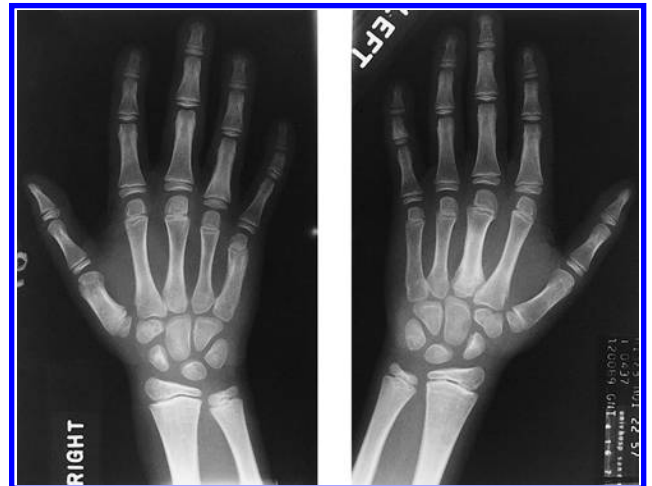


Figure 79.13 Roentgenogram of the hands of the same 16-year-old looks normal. This highlights the difference in the bones of this disease, from those of disorders with more severe dysostosis multiplex.

with I cell disease, or mucopolipidosis II and Gm1 gangliosidosis, and Hurler disease have the most prominent bony changes. Computed tomography (CT) scans or magnetic resonance imaging (MRI) may reveal mild atrophy early, but this is progressive with the neurodegeneration.

Blood smears may reveal the presence of metachromatic inclusion bodies in the lymphocytes. They are characteristically coarser and sparser than those seen in Hurler disease. Inclusions may also be seen in cells of the bone marrow. Chondrocytes in cartilage biopsied from the iliac crest and the ribs have been reported to be

vacuolated [32]. The diagnosis of a mucopolysaccharidosis is first made by the findings of increased quantities of mucopolysaccharide in the urine. In this disorder, it is heparan sulfate that is excreted in excess [33].

Patients with this disorder accumulate gangliosides in the brain [34, 35] including Gm2 and Gm3 [34] or there may be increased amounts of Gm1 [35]. The electron microscopic appearance of the neurons may be like those of Tay-Sachs disease [34] ([Chapter 90](#)). There may also be zebra bodies, and mucopolysaccharide may accumulate in the brain, as well as in the peripheral tissues.

GENETICS AND PATHOGENESIS

All four types of Sanfilippo syndrome are transmitted in an autosomal recessive fashion. Multiple affected siblings have been observed in several families, and consanguinity has been documented [18]. The frequency of the disease has been estimated at one in 24,000 in the Netherlands [18, 36]. In this population and in Great Britain [37], the most frequent type was A. In the Cayman Islands there is a very high prevalence of Sanfilippo A disease [22]; the carrier frequency is 0.1. In Greece, ten of 11 patients reported were of type B [38].

The heparan sulfate molecule (Figure 79.1) consists of a series of glucuronic acid and iduronic acid molecules alternating with glucosamine residues [39]. The amino nitrogen of the glucosamine moiety may be either sulfated or acetylated, and the 6-hydroxyl may be sulfated. The stepwise degradation of heparan sulfate provides the sites for the defects in the various forms of Sanfilippo disease. Heparan-N-sulfatase, the site of the defect in Sanfilippo disease type A [6, 40, 41], catalyzes the breakdown of the molecule by splitting off the sulfate groups linked to the amino group of glucosamine. The enzyme has been isolated and purified [42] and is formally a sulfamate sulfohydrolase. α -N-acetylglucosaminidase, the defective enzyme in type B, catalyzes heparan sulfate breakdown at the glucosamine to hexuronic acid linkage removing the N-acetylglucosamine generated by acetyl transfer in the IIIC reaction. This enzyme has been purified from human liver and urine, and the biosynthesis of the mature lysosomal enzyme has been elucidated [43–45]. In the sequential catabolism of heparan sulfate, removal of the sulfate in the reaction catalyzed by heparan-N-sulfatase exposes a terminal glucosamine moiety. This cannot be cleaved until it is acetylated, after which the reaction deficient in type B Sanfilippo disease comes into play. The acetylation is catalyzed by a specific N-acetyltransferase, and it is this reaction that is defective in type C Sanfilippo disease [9, 46, 47]. This is a two-step reaction in which the enzyme is first acetylated on the cytoplasmic side of the membrane and then transfers this acetyl group now inside the lysosome to a glucosamine. AcetylCoA does not cross the lysosomal membrane. Some patients with type C disease are defective in the second step and others in both steps [48–50].

The defective enzyme in type D Sanfilippo disease is in α -N-acetylglucosamine-6-sulfatase [50, 51]. Glucosamine residues in heparan sulfate can be either N-sulfated or O-sulfated, and there are specific sulfatases for each residue. Defective activity of either leads to accumulation and to the Sanfilippo syndrome. The glucosamine-6-sulfatase has been purified [52] and its cDNA has been cloned [11]. Its structure is homologous to other sulfatases. Immunoprecipitation studies have demonstrated cross-reactive material in the Sanfilippo B-syndrome [53]. The enzyme is also involved in the degradation of keratan sulfate, but patients do not excrete keratan sulfate, because

this block may be obviated by other enzymes. Patients excrete N-acetylglucosamine sulfate, as well as heparan sulfate [54].

Within each of the forms, there is not only interfamilial variability, but also intrafamilial variability [55, 56]. Patients with all of the forms excrete heparan sulfate and no other glucosaminoglycan.

Detection of heterozygotes has been accomplished through enzyme assay [57] and by ^{35}S accumulation, which may be more reliable in distinguishing heterozygotes. Positive identification by enzyme assay is accurate, but there may be overlap, making identification of the normal noncarrier individual unreliable. When the mutation is known, molecular methods may be used for the detection of carriers.

Intrauterine diagnosis of the affected fetus has been accomplished in types A and B disease through assay of the relevant enzyme in amniotic cells in culture [58]. It may be useful to confirm the assay by assessment of ^{35}S accumulation, because some heterozygotes have very low levels of enzyme in the usual assays. Chorionic villus material has abundant enzyme activity and is available for the prenatal diagnosis of each of the forms of Sanfilippo disease [58–61].

The gene that encodes the heparan-N-sulfatase, defective in MPSIIIA, has eight exons over 11 kb [13, 62]. Among the early mutations identified was an 11 bp deletion [13]. Some other deletions have been reported, but most mutations have been missense [15, 16, 63–66]. Among Australian, Dutch, and German patients, the most common mutation was R245H [64]. Among Polish patients, it was R74C [65], S66W in Sardinians [65], and 1091delC in Spaniards [66].

The gene for the acetylglucosaminidase defective in MPSIIIB contains six exons over 8.5 kb [14]. A number of mutations have been identified [14, 16, 67, 68]. A number of replacements of arginine by histidine or stop codon were found at CPG hotspots [14].

TREATMENT

There is no effective treatment for Sanfilippo patients. Enzyme replacement therapy has been attempted using partially purified enzyme, leukocytes, and cultured fibroblasts [69], after which there may have been some changes in urinary mucopolysaccharides, but clinical benefit has not been evident. Bone marrow transplantation has been explored in Sanfilippo disease, as well as in other mucopolysaccharidoses, but results have not been impressive [70] in the Sanfilippo group, and the procedure is not recommended. The overwhelming experience with MPSIII is that transplantation of marrow or stem cells does not reverse the inexorable neurodegeneration of this disease. Loperamide hydrochloride may be useful in the management of diarrhea. The dose is 1–2 mg up to four times a day.

Behavioral modification may be useful in the

management of problems of behavior. Otherwise, therapy is supportive.

REFERENCES

- Harris RC. Mucopolysaccharide disorder: a possible new genotype of Hurler's syndrome. *Am J Dis Child* 1961; **102**: 741 (Abstr.).
- Sanfilippo SJ, Good RA. Urinary acid mucopolysaccharides in the Hurler syndrome and Morquio's disease. *J Pediatr* 1962; **61**: 296.
- Sanfilippo SJ, Podosin R, Langer L, Good RA. Mental retardation associated with acid mucopolysacchariduria (heparitin sulfate type). *J Pediatr* 1963; **63**: 837 (Abstr.).
- Leroy JG, Crocker AC. Clinical definition of the Hurler-Hunter phenotypes. A review of 50 patients. *Am J Dis Child* 1966; **112**: 518.
- Kresse H, Wiesmann U, Gantz M *et al.* Biochemical heterogeneity of the Sanfilippo syndrome: preliminary characterization of two deficient factors. *Biochem Biophys Res Commun* 1971; **42**: 892.
- Kresse H, Neufeld EF. The Sanfilippo A corrective factors. *J Biol Chem* 1972; **247**: 2164.
- O'Brien JS. Sanfilippo syndrome: profound deficiency of alpha-acetylglucosaminidase activity in organs and skin fibroblasts from type B patients. *Proc Natl Acad Sci USA* 1972; **69**: 1720.
- Von Figura K, Kresse H. The Sanfilippo B corrective factor: a N-acetyl- α -D-glucosaminidase. *Biochem Biophys Res Commun* 1972; **48**: 262.
- Klein U, Kresse H, von Figura K. Sanfilippo syndrome type C: deficiency of acetyl-CoA: α -glucosaminide N-acetyl-transferase in skin fibroblasts. *Proc Natl Acad Sci USA* 1978; **75**: 5185.
- Kresse H, Paschke E, von Figura K *et al.* Sanfilippo disease type D: deficiency of N-acetylglucosamine 6-sulfate sulfatase required for heparan sulfate degradation. *Proc Natl Acad Sci USA* 1980; **77**: 6822.
- Robertson DA, Freeman C, Nelson PV *et al.* Human glucosamine-6-sulfatase cDNA reveals homology with steroid sulfatase. *Biochem Biophys Res Commun* 1988; **157**: 218.
- Robertson DA, Callen DF, Baker EG *et al.* Chromosomal localization of the gene for human glucosamine 6-sulphatase to 12q14. *Hum Genet* 1988; **79**: 175.
- Scott HS, Blanch L, Guo X-H *et al.* Cloning of the sulphamidase gene and identification of mutations in Sanfilippo A syndrome. *Nat Genet* 1995; **11**: 465.
- Zhao HG, Li HH, Bach G *et al.* The molecular basis of Sanfilippo syndrome type B. *Proc Natl Acad Sci USA* 1996; **93**: 6101.
- Blanch L, Weber B, Guo XH *et al.* Molecular defects in Sanfilippo syndrome type A. *Hum Mol Genet* 1997; **6**: 787.
- Schmidtchen A, Greenberg D, Zhao HG *et al.* NAGLU mutations underlying Sanfilippo syndrome type B. *Am J Hum Genet* 1998; **62**: 64.
- McKusick VA. *Heritable Disorders of Connective Tissue*, 4th edn. St Louis: CV Mosby, 1972: 521.
- Van de Kamp JJP, Niermeijer MF, von Figura K, Giesberts MAH. Genetic heterogeneity and clinical variability in the Sanfilippo syndrome (types A, B and C). *Clin Genet* 1981; **20**: 152.
- Lindor NM, Hoffman A, O'Brien JF *et al.* Sanfilippo syndrome type A in two adult sibs. *Am J Med Genet* 1994; **53**: 241.
- Nidiffer FD, Kelly TE. Developmental and degenerative patterns associated with cognitive behavioral and motor difficulties in the Sanfilippo syndrome: an epidemiological study. *J Ment Defic Res* 1987; **27**: 185.
- Van Schrojenstein-DeValk HMJ, van de Kamp HP. Follow-up on seven adult patients with mild Sanfilippo B disease. *Am J Med Genet* 1987; **28**: 125.
- Matalon R, Deanching M, Nakamura F, Bloom A. A recessively inherited lethal disease in a Caribbean isolate – a sulfamidase deficiency. *Pediatr Res* 1980; **14**: 524.
- McDowell GA, Cowan TM, Blitzer MG, Greene CL. Intrafamilial variability in Hurler syndrome and Sanfilippo syndrome type A: implications for evaluation of new therapies. *Am J Med Genet* 1965; **47**: 1092.
- Di Natale P. Sanfilippo B disease: a re-examination of a particular sibship after 12 years. *J Inherit Metab Dis* 1991; **14**: 23.
- Sewell AC, Pontz BF, Benischek G. Mucopolysaccharidosis type IIIC (Sanfilippo): early clinical presentation in a large Turkish pedigree. *Clin Genet* 1988; **34**: 116.
- Coppa GV, Giorgi PL, Felici L *et al.* Clinical heterogeneity in Sanfilippo disease (mucopolysaccharidosis III) type D: presentation of two new cases. *Eur J Pediatr* 1983; **140**: 130.
- Kaplan P, Wolfe LS. Sanfilippo syndrome type D. *J Pediatr* 1987; **110**: 267.
- Siciliano L, Fiumara A, Pavone L *et al.* Sanfilippo syndrome type D in two adolescent sisters. *J Med Genet* 1991; **28**: 402.
- Herd JK, Subramanian S, Robinson H. Type III mucopolysaccharidosis: report of a case with severe mitral valve involvement. *J Pediatr* 1973; **82**: 1011.
- Tylki-Szymanska A, Metera M. Precocious puberty in three boys with Sanfilippo A (mucopolysaccharidosis III A). *J Pediatr Endocrinol Metab* 1995; **8**: 291.
- Spranger J, Teller W, Kosenow W *et al.* Die HS-mucopolysaccharidose von Sanfilippo (Polydystrophie oligophrenie). Bericht Über 10 Patienten. *Z Kinderheilk* 1967; **101**: 71.
- Silberberg R, Rimoin DL, Rosenthal RE, Hasler MB. Ultrastructure of cartilage in the Hurler and Sanfilippo syndromes. *Arch Pathol* 1972; **94**: 500.
- Gordon BA, Haust MD. The mucopolysaccharidoses types I II and III: urinary findings in 23 cases. *Clin Biochem* 1970; **3**: 302.
- Wallace BJ, Kaplan D, Adachi M *et al.* Mucopolysaccharidosis type III. Morphologic and biochemical studies of two siblings with Sanfilippo syndrome. *Arch Pathol* 1966; **82**: 462.
- Dekaban AS, Patton VM. Hurler's and Sanfilippo's variants of mucopolysaccharidosis. *Arch Pathol* 1971; **91**: 434.
- Van de Kamp JJP. The Sanfilippo syndrome: a clinical and genetical study of 75 patients in the Netherlands (doctoral thesis). S-Gravenhage, Rasmans JH, 1979.
- Whiteman P, Young E. The laboratory diagnosis of Sanfilippo disease. *Clin Chim Acta* 1970; **76**: 139.

38. Beratis NG, Sklower SL, Wilbur L, Matalon R. Sanfilippo disease in Greece. *Clin Genet* 1986; **29**: 129.
39. Roden L. Structure and metabolism of connective tissue proteoglycans. In: Lennarz WH (ed.). *The Biochemistry of Glycoproteins and Proteoglycans*. New York: Plenum Press, 1980: 267.
40. Kresse H. Mucopolysaccharidosis III A (Sanfilippo A disease): deficiency of heparin sulfamidase in skin fibroblasts and leucocytes. *Biochem Biophys Res Commun* 1973; **54**: 1111.
41. Matalon R, Dorfman A. Sanfilippo A syndrome: sulfamidase deficiency in cultured skin fibroblasts and liver. *J Clin Invest* 1974; **54**: 907.
42. Freeman C, Hopwood JJ. Human liver sulphamate sulphohydrolase. *Biochem J* 1986; **234**: 83.
43. Von Figura K. Human α -N-acetylglucosaminidase. I Purification and properties. *Eur J Biochem* 1977; **80**: 525.
44. Sasaki T, Sukegawa K, Masue M *et al.* Purification and partial characterization of α -N-acetylglucosaminidase from human liver. *J Biochem* 1991; **110**: 842.
45. Von Figura K, Hasilik A, Steckel F, van de Kamp J. Biosynthesis and maturation of α -N-acetylglucosaminidase in normal and Sanfilippo B fibroblasts. *Am J Hum Genet* 1984; **36**: 93.
46. Bartsocas C, Grobe H, Vande Kamp JJP *et al.* Sanfilippo type C disease clinical findings in four patients with a new variant of mucopolysaccharidosis III. *Eur J Pediatr* 1979; **130**: 251.
47. Kresse H, von Figura K, Klein U. New biochemical subtype of the Sanfilippo syndrome: characterization of the storage material in cultured fibroblasts of Sanfilippo C patients. *Eur J Biochem* 1978; **92**: 333.
48. Bame KJ, Rome LH. AcetylCoA: α -glucosaminide N-acetyl transferase from rat liver. *Methods Enzymol* 1987; **138**: 607.
49. Bame KJ, Rome LH. Genetic evidence for transmembrane acetylation by lysosomes. *Science* 1986; **233**: 1087.
50. Kresse H, Paschke E, von Figura K *et al.* Sanfilippo disease type D; deficiency of N-acetylglucosamine-6-sulfatase required for heparan sulfate degradation. *Proc Natl Acad Sci USA* 1980; **77**: 6622.
51. Freeman C, Hopwood JJ. Human glucosamine-6-sulphatase deficiency. Diagnostic enzymology towards heparin-derived trisaccharide substrates. *Biochem J* 1992; **282**: 605.
52. Freeman C, Clements PR, Hopwood JJ. Human liver N-acetylglucosamine-6-sulphate sulphatase. Purification and characterization. *Biochem J* 1987; **246**: 347.
53. Von Figura K, Kresse H. Sanfilippo disease type B: presence of material cross reacting with antibodies against α -N-acetylglucosaminidase. *Eur J Biochem* 1976; **61**: 581.
54. Fuchs W, Beck M, Kresse H. Intralysosomal formation and metabolic fate of N-acetylglucosamine 6-sulfate from keratan sulfate. *Eur J Biochem* 1985; **151**: 551.
55. Andria G, Di Natale P, Del Giudice E *et al.* Sanfilippo B syndrome (MPS III): mild and severe forms within the same sibship. *Clin Genet* 1979; **15**: 500.
56. Van de Kamp JJP, van Pelt JF, Liem KO *et al.* Clinical variability in Sanfilippo B disease: a report of six patients in two related sibships. *Clin Genet* 1976; **10**: 279.
57. Toone JR, Applegarth DA. Carrier detection in Sanfilippo A syndrome. *Clin Genet* 1988; **33**: 401.
58. Kleijer WJ, Janse HC, Vosters RPL *et al.* First trimester diagnosis of mucopolysaccharidosis IIIA (Sanfilippo A disease). *N Engl J Med* 1986; **314**: 185.
59. Di Natale P, Pannone N, D'Argenio G *et al.* First trimester prenatal diagnosis of Sanfilippo C disease. *Prenat Diagn* 1987; **7**: 603.
60. Poenaru L. First trimester prenatal diagnosis of metabolic diseases: a survey of countries from the European Community. *Prenat Diagn* 1987; **7**: 333.
61. Nowakowski RW, Thompson JN, Taylor KB. Sanfilippo syndrome type D: a spectrophotometric assay with prenatal diagnostic potential. *Pediatr Res* 1989; **26**: 462.
62. Karageorgos LE, Guo X-H, Blanch L *et al.* Structure and sequence of the human sulphamidase gene. *DNA Res* 1996; **3**: 269.
63. Weber B, Guo XH, Wraith JE *et al.* Novel mutations in Sanfilippo A syndrome: implications for enzyme function. *Hum Mol Genet* 1997; **6**: 1573.
64. Bunge S, Ince H, Steglich C *et al.* Identification of 16 sulfamidase gene mutations including the common R74C in patients with mucopolysaccharidosis type IIIA (Sanfilippo A). *Hum Mutat* 1997; **10**: 479.
65. Di Natale P, Balzano N, Esposito S, Villani GR. Identification of molecular defects in Italian Sanfilippo A patients including 13 novel mutations. *Hum Mutat* 1998; **11**: 313.
66. Montfort M, Vilageliu L, Garcia-Giralt N *et al.* Mutation 1091delC is highly prevalent in Spanish Sanfilippo syndrome type A patients. *Hum Mutat* 1998; **12**: 274.
67. Zhao HG, Aronovich EL, Whitley CB. Genotype-phenotype correspondence in Sanfilippo syndrome type B. *Am J Hum Genet* 1998; **62**: 53.
68. Coll MJ, Antón C, Chabás A. Allelic heterogeneity in Spanish patients with Sanfilippo disease type B. Identification of eight new mutations. *J Inher Metab Dis* 2001; **24**: 83.
69. Munnich A, Saudubray JM, Hors-Cayla MC *et al.* Letter to the Editor: Enzyme replacement therapy by transplantation of HLA-compatible fibroblasts in Sanfilippo syndrome: another trial. *Pediatr Res* 1982; **16**: 259.
70. Shapiro EG, Lockman LA, Balthazor M, Krivit WJ. Neuropsychological outcomes of several storage diseases with and without bone marrow transplantation. *J Inher Metab Dis* 1995; **18**: 413.

Morquio syndrome/mucopolysaccharidosis type IV/keratan sulfaturia

Introduction	588	Treatment	594
Clinical abnormalities	589	References	594
Genetics and pathogenesis	593		

MAJOR PHENOTYPIC EXPRESSION

Shortness of stature, pectus carinatum, dorsolumbar kyphosis, odontoid hypoplasia, genu valgum, corneal clouding, dental anomalies, aortic valve disease, and keratan sulfaturia. Activity of galactosamine-6-sulfate sulfatase is deficient in type A Morquio syndrome; lysosomal β -galactosidase is defective in type IV B.

INTRODUCTION

The syndrome was described by Morquio in 1929 [1] in four affected siblings in Uruguay who were the products of a marriage of first cousins of Swedish origin. In the same year, Brailsford [2] described a similar patient in England. The excretion of keratan sulfate in the urine is the defining biochemical feature of patients with this disease, and keratan sulfate has been documented to accumulate in tissues [3–5]. Defective degradation of keratan sulfate leads to its accumulation in those tissues in which it is normally abundant: cartilage, nucleus pulposus and cornea – tissues that are prominent in the clinical manifestation of the disease. Keratan sulfate consists of alternating galactose and N-acetylglucosamine residues; each may be sulfated. The molecular defect in type A or classic Morquio syndrome is in N-acetylgalactosamine-6-sulfatase encoded by the *GALNS* gene. It is also a galactose-6-sulfatase responsible for the cleavage of the galactose-6-sulfate moieties of keratan sulfate (Figure 80.1) [6–8]. This enzyme also catalyzes the removal of sulfate moieties from N-acetylgalactosamine-6-sulfate residues that are present in chondroitin-6-sulfate (C6S), and this leads to excretion in excess of chondroitin sulfate in Morquio syndrome. The accumulation of the KS and C6S, in bone and cornea leads to a systemic skeletal chondrodysplasia [9].

In the sequential degradation of keratan sulfate, once the first sulfate has been cleaved, the terminal galactose is

cleaved in a reaction catalyzed by β -galactosidase encoded by the *GLB1* gene. This is the enzyme that is defective in type B Morquio syndrome [10–12].

The gene for the galactose-6-sulfatase deficient in type A Morquio disease has been cloned [13] and mapped to chromosome 16q 24.3 [14, 15]. A number of mutations has been described [16–19]. The gene for the β -galactosidase defective in type B has been localized to chromosome 3 p21-cen [20]. Some mutations have been defined [21].

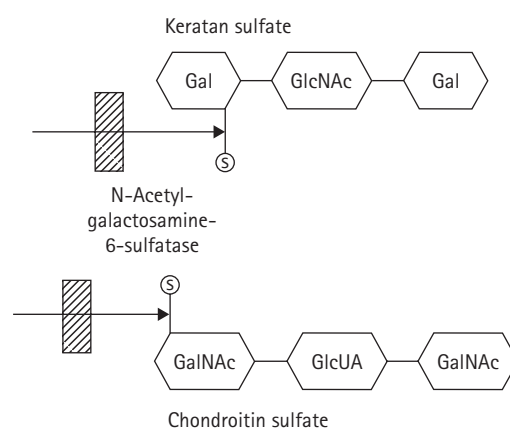


Figure 80.1 N-Acetylgalactosamine-6-sulfatase and the degradation of keratan sulfate. This is the site of the defect in type A Morquio syndrome. The enzyme also hydrolyzes the sulfate from the N-acetylgalactosamine-6-sulfate moieties that occur in chondroitin-6-sulfate.

CLINICAL ABNORMALITIES

The clinical pictures in types A and B are indistinguishable. In both, there is considerable heterogeneity ranging from mild to severe, including even hydrops fetalis phenotypes. The most characteristic features of this syndrome are skeletal deformities and shortness of stature, which is particularly short-trunked, though the long bones are also involved. The neck is short and the head appears to sit directly on the barrel chest, which classically has a very pronounced pectus carinatum (Figures 80.2, 80.3, 80.4, 80.5, 80.6, 80.7, and 80.8). The upper part of the sternum



Figure 80.2 A 16-year-old patient with Morquio disease type A. He had normal intelligence. Illustrated are the typical pectus carinatum, deformed arms and legs, and stunted stature.



Figure 80.3 FD: A ten-year-old Honduran boy with Marquio syndrome. He was short and had a prominent pectus carinatum. The neck appeared very short.

may be almost horizontal. There is also a pronounced genu valgum, and patients often have a semi-crouching stance. The joints are enlarged and prominent (Figures 80.9, 80.10, 80.11, 80.12, 80.13, and 80.14). On the other hand, as a result of ligamentous laxity, there is usually extreme hypermobility and hyperextension of the joints, particularly at the wrists, where there may be marked ulnar deviation (Figure 80.10). Joints may become stiff with age. Pes planus is also seen.

These skeletal changes are not obvious during the first year of life because intrauterine growth and early extrauterine development are normal. Prominence of the lower ribs may first bring the patient in for medical consultation at 12 to 18 months of age. Others first come to attention because of prominence of the sternum. Flat feet may be an early sign. In the second or third year, patients develop awkward gaits and impaired growth as skeletal deformities begin to be evident. The deformities are progressive and become exaggerated with age [22]. Patients with milder disease have presented in early adolescence



Figure 80.4 FD: In the lateral, the kyphosis and pectus are seen clearly. There was irregular flaring at the rib cage.



Figure 80.5 MKMT: A four-year-old with Morquio type II disease. The prominent pectus is shown.



Figure 80.6 ABV: A 12-year-old boy with Morquio disease. He had kyphosis, as well as a prominent manubrium and a small thoracic cage. He had marked genu valgum and flexion deformities of the hip. Activity of N-acetylgalactosamine-6-sulfatase in fibroblasts was undetectable.



Figure 80.8 FRAM: A 26-year-old with Morquio disease and a spastic tetraplegia, a consequence of cord compression at C1 and C2 and odontoid hypoplasia.



Figure 80.7 MAZ: A three-year-old girl with Morquio disease and the typical pectus deformity, short neck, and flat facies, as well as valgus deformities at the knees and ankles.



Figure 80.9 ABU: The genu valgum. The ankles and feet were quite broad.

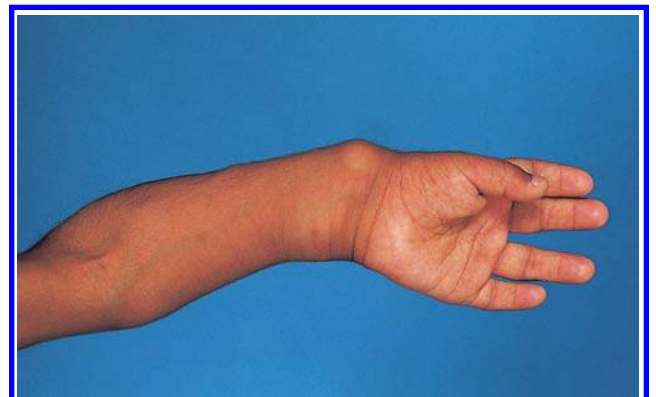


Figure 80.10 ABU: The wrists were very floppy and he had poor grip strength.



Figure 80.11 ABAO: The wrists of this 14-year-old patient were very floppy, and ulnar deviation was the result of the very short ulna.

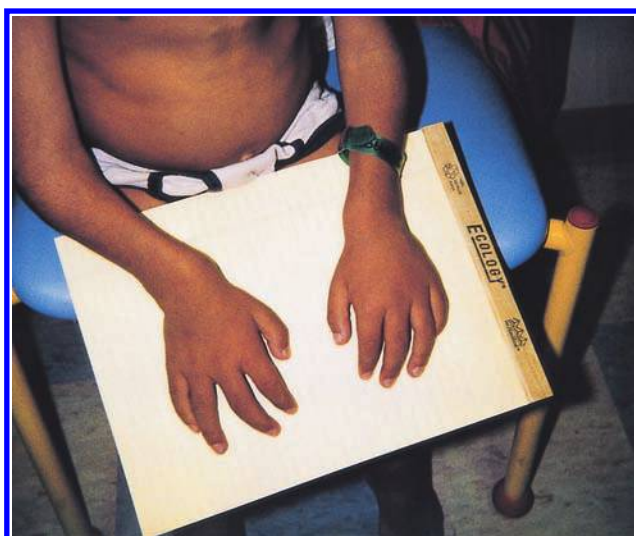


Figure 80.12 FD: The joints of the wrist and hand were enlarged.

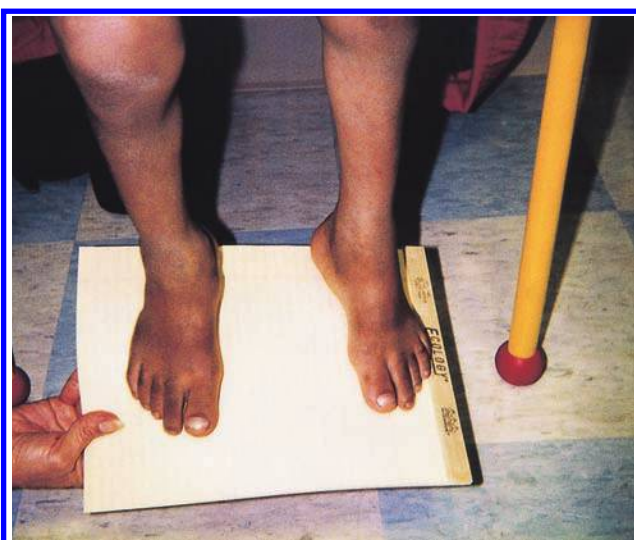


Figure 80.13 FD: The ankles and feet were also broadened.

with bilateral Legg-Perthes disease [23]. In similar fashion, a 30-year-old was reported with severe hip disease [24]. Growth is markedly slowed after about five years of age. Maximal height of 85–100 cm is usually reached by seven to eight years of age. Diagnostic delay is the rule and the diagnosis may not be made until as late as 15 years [25, 26].

These patients have fine corneal opacities, which are usually visible only on slit lamp examination, but may cause a hazy cloudiness of the cornea [3, 27]. Glaucoma was observed in siblings in their thirties [28]. Progressive sensorineural or mixed deafness usually begins in the second decade and is present uniformly after 20 years [29].

The mouth tends to be broad, and there may be spacing between the teeth which may be small and flared [30, 31]. The enamel is hypoplastic both in deciduous and in permanent teeth. The teeth develop a gray or yellowish color, and the enamel becomes flaky or fractured. Molars are tapered and often have sharp cusps. The teeth easily develop caries. In one series [32], dental changes were observed only in type A, not in type B. A regular later manifestation of the disease is aortic regurgitation [33]. Severe scoliosis may lead to cardiorespiratory complications. Three of Morquio's original patients died of pulmonary complications. Inguinal hernia is probably more common than in normal individuals. The brain is normal and patients usually have normal intelligence. Ian Smith, a three feet tall, 14-year-old with Morquio syndrome played the title role in the Disney film *Simon Birch* and speaks publicly in support of rare diseases. Facial features may be somewhat coarse, the chin prominent and the mouth wide. The neck is short (Figures 80.2 and 80.3). Roentgenographic findings (Figures 80.15, 80.16, and 80.17) in Morquio disease vary with the age of the patient [34]. The most characteristic and consistent finding is the universal platyspondyly or vertebra plana, which produces the short spine. The vertebral bodies are usually oval-shaped in the younger affected child, becoming flatter and more rectangular in later childhood and flat in the adult. The cervical spine is striking in that the odontoid process of C2 is either absent or hypoplastic [35]. The remainder of the cervical vertebrae are flat. The thoracic and lumbar vertebrae show flattening and anterior beaking or tonguing. L1 is often short, anteriorly wedged, and displaced posteriorly, accounting for the gibbus. Patients with this syndrome always have a marked coxa valga deformity (Figure 80.15). The pelvis is narrow. With age, the anterior portions of the ribs become wide and spatula-shaped. The sternum protrudes. The femoral head becomes progressively flattened and fragmented; it may be completely resorbed. The femoral neck initially loses its angle and later becomes thickened. The distal femur is wide, as is the proximal tibia. These changes contribute to the production of the genu valgum that is characteristic of this disease. The distal end of the humerus is wide and irregular, as are the proximal ulna and radius – changes similar to the corresponding bones of the lower extremities. The growth plates of the distal ulnae and radii are slanted toward each other, the ulna usually being somewhat shorter than the

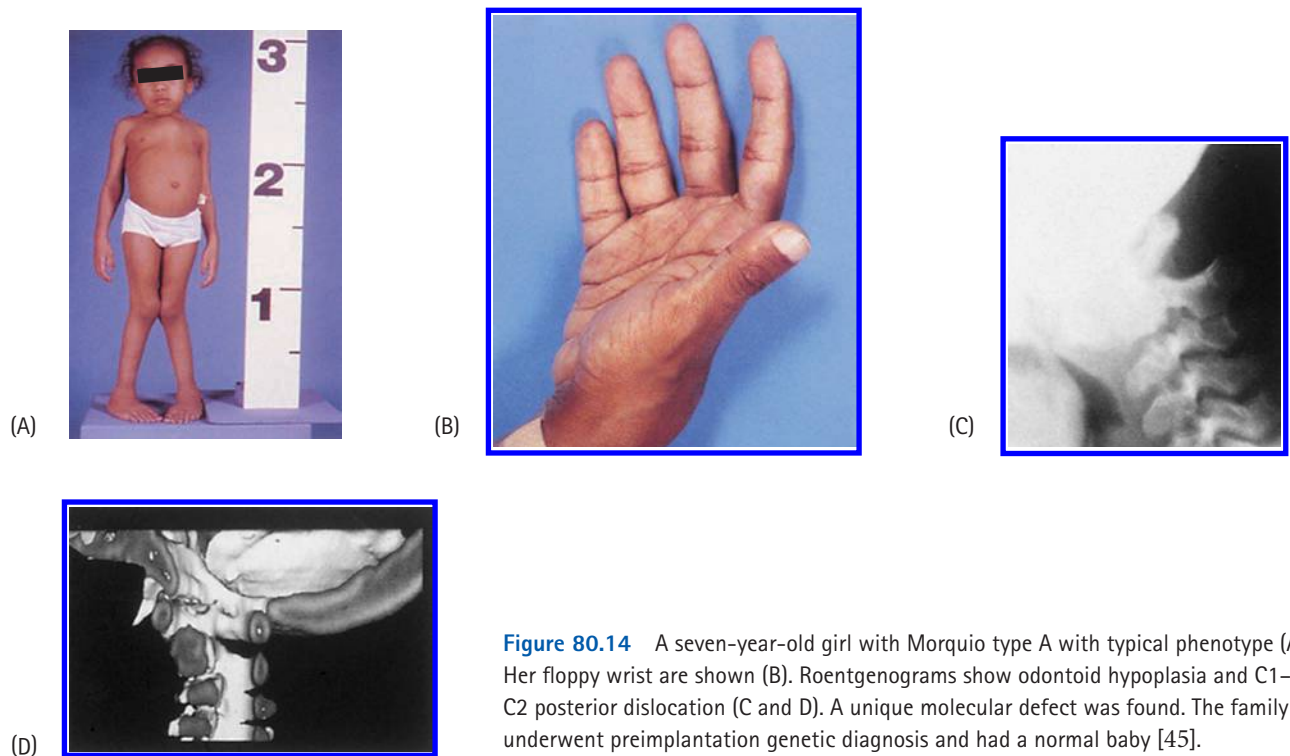


Figure 80.14 A seven-year-old girl with Morquio type A with typical phenotype (A). Her floppy wrist are shown (B). Roentgenograms show odontoid hypoplasia and C1–C2 posterior dislocation (C and D). A unique molecular defect was found. The family underwent preimplantation genetic diagnosis and had a normal baby [45].



Figure 80.15 Roentgenogram of the spine of a five-year-old boy with Morquio syndrome. The vertebral bodies were very flat and beaked anteriorly. The second vertebra from the top was hypoplastic and displaced posteriorly. This is the genesis of the gibbus in this syndrome. (Illustration was kindly provided by Dr David Rimoin, University of California, Los Angeles and Cedars of Lebanon Hospital, Los Angeles, California.)



Figure 80.16 Roentgenogram of the pelvis of the same patient. The capital femoral epiphyses were flattened and irregular. There was coxa valga. The lateral margins of the acetabula were hypoplastic, creating, in essence, large acetabula extending to the anterior superior iliac spine. (Illustration was kindly provided by Dr David Rimoin.)

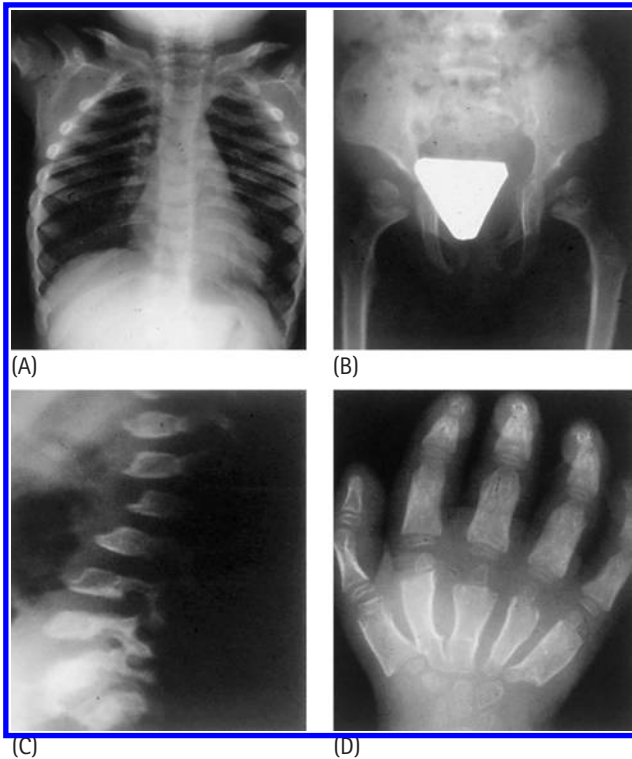


Figure 80.17 The bony abnormalities involved spatulate ribs (A), the typical pelvis with flattened femoral epiphyses and hypoplastic acetabular lateral margins and coxa valga (B). Posterior scalloping of the vertebrae and gibbus deformity are shown (C). Roentgenogram of the hand illustrated flattened carpal bones and proximal tapering of flexed metacarpals (D).

radius. The ossified carpal bones are small and may be reduced in number. The metacarpals are short and their distal metaphyses are widened. Osteoporosis is common in the adult patient.

Ocular manifestations of disease in 20 patients include corneal opacification (13/20), astigmatism (12/20), and cataract (6/20) [36]. Visual acuity improved with ophthalmologic correction.

A very dangerous complication of the bony deformity in this syndrome is that the spinal cord may be compressed following atlantoaxial subluxation or dislocation [12, 37–39]. This is a major cause of death. Manipulation of the head for intubation may be particularly risky in these patients for this reason and anesthesia should be planned with this problem in the forefront, but subluxation may occur even during sleep and lead to death. This propensity for subluxation is attributed to the hypoplasia of the odontoid process and to the general laxity of the ligaments. These features are present in all patients with the disease, who therefore sooner or later may all expect to experience a complication of compression of the spinal cord. Neurologic manifestations may include weakness and difficulty in walking, uselessness of the legs on awakening, or spastic paraplegia (Figure 80.8). Loss of vibratory sensation in the lower extremities may be an early sign,

and many patients have hyperactive deep tendon reflexes. Spinal cord compression may also occur at the level of the thoracolumbar gibbus. Subluxation of vertebra C2 to C3, as well as C1 to C2, has been observed [40] in type B patients. Conjunctival biopsy may show intracytoplasmic vacuoles indicating lysosomal storage [41]. Metachromatic granules may be seen in the polymorphonuclear leukocytes or cultured fibroblasts [42]. Storage vacuoles have been demonstrated in the skin and in chondrocytes [43]. There is minimal evidence of storage in the brain. Generally, mucopolysaccharidosis (MPS) IVA patients with a severe form do not survive beyond the third decade of life, whereas those patients with an attenuated form may survive over years [9].

GENETICS AND PATHOGENESIS

Morquio syndrome is transmitted by autosomal recessive genes. It has been reported on several occasions that normal couples have produced multiple involved siblings. Parental consanguinity has been documented [22]. Prenatal diagnosis may be performed by the assay of either enzyme in cultured amniocytes or chorionic villus tissue [44]. An effective preventive strategy for Morquio disease and other lysosomal storage disorders is preimplantation genetic diagnosis which has been carried out on 3-day embryos in Saudi Arabia with an almost 90 percent success rate [45].

Patients with Morquio disease characteristically have increased concentrations of keratan sulfate in the urine [46]. Levels of the acid mucopolysaccharide, which does not contain uronic acid, are often two to three times the normal amount. With age, the concentrations of this compound in urine decrease. An immunoassay for keratan sulfate is capable of diagnostic assay of either blood or urine in any age group and permits distinction of mild and severe phenotypes [47]. More recently [48], a liquid chromatography tandem mass spectrum method has been developed for the assessment of keratan sulfate levels in blood. Levels varied with age, as well as severity of disease. The method is useful for diagnosis and the evaluation of severity of disease. Absence of keratan sulfaturia has been documented in enzyme-proven type B [11] and type A [23, 24, 32] Morquio disease.

In Morquio type A, the defective enzyme catalyzes the removal of 6-sulfate moieties from galactose, and from the N-acetylgalactosamine residues of chondroitin sulfate. This latter property gave the enzyme its name. N-acetylgalactosamine-6-sulfatase has been purified from human placenta, and the defective enzyme has been demonstrated in cultured fibroblasts and in brain [3, 5, 8]. Deficiency of the type A enzyme has been demonstrated in patients not excreting keratan sulfate [49]. In five patients studied immunochemically, no cross-reacting material was demonstrated [50], but cross-reactive material has been demonstrated immunochemically in both Morquio type B and type A [51]. The full length of cDNA has been cloned

and sequenced for human N-acetylgalactosamine-6-sulfatase [13], and transfection into deficient fibroblasts led to activity. The type A gene has 14 exons, and the sequence of 522 amino acids of the enzyme has considerable homology with other sulfatases, such as iduronate-2-sulfatase. At least 16 polymorphisms have been identified in the *GALNS* gene [52]. Polymorphic haplotypes may be employed for carrier detection and prenatal diagnosis in informative families [52], and this may be useful when mutations have not been identified. A considerable number and variety of mutations have been found in the Morquio type A gene [53]. Most have been missense point mutations, but there were a few nonsense and splice site mutations and small deletions. Insertions and large rearrangements were rare. Severe disease was present in patients with a T to C change at nucleotide 468 resulting in V138 A and a C to T transition at 386 substituting a cysteine for arginine 386 [18]. A two-base deletion at 1342delCA was also associated with severe disease [54]. I113F was found to be a common missense mutation in Caucasian, particularly in Irish patients [16, 53], as was T312S. Another novel mutation c.1567T>G was found in a Chinese patient located in the termination codon TAG. This change of TAG to GAG extended the peptide chain by 92 amino acids; marked change in the protein structure led to an impressive reduction in enzyme activity [55]. In 24 unrelated Chinese patients with Morquio type A disease, 27 mutations were found [54], of which 16 were novel; there were two splicing mutations (c.567-1G>T and c.634-1G>A), two nonsense mutations (p.W325X and p.Q422X), and 12 missense mutations (p.T881, p.H142R, p.P163H, p.G168L, p.H236D, p.N289S, p.T312A, p.G316V, p.A324E, p.L366P, p.Q422K, and p.F452L). The p.G340D mutation was common, accounting for 16.7 percent of Chinese mutant alleles [56].

Some mutations in the β -galactosidase gene have been identified in genetic compounds [57, 58]. Depending on the mutation, the phenotype can vary from that of severe GM1 gangliosidosis (Chapter 89) to Morquio disease type B [21]. In a patient with Morquio B disease, p.Y333H was found on one allele [59]; p.R201H was on the other. The latter allele has been associated with poorly transported protein products through the endoplasmic reticulum.

The diagnosis is best made by assay of cultured fibroblasts or leukocytes using a substrate derived from chondroitin-6-sulfate for the sulfatase [60, 61] and using p-nitrophenyl- or 4-methylumbelliferyl- β -galactoside for the β -galactosidase. Enzyme activity may be assessed by tandem mass spectrometry of blood spots and used in programs of newborn screening [62]. Novel specific substrates have been developed for newborn screening for Morquio A disease, and also for Maroteaux-Lamy disease [63].

In addition to types A and B, there are other clinical examples of Morquio syndrome, usually mild, in which defects in neither of these enzymes can be detected. These are non-keratan sulfate-excreting patients [64].

The skeletal deformities and other symptoms in these patients are similar to those seen in Morquio syndrome, but less severe. There is platyspondyly, genu valgum, flat feet, pectus carinatum, and flat, fragmented femoral heads. The pathogenesis of disease is not explained simply by the storage of material in chondrocytes. It has been proposed that accumulation in macrophages within cartilage canals and inadequate regression of canals could contribute to cartilaginous disease [65].

TREATMENT

Surgical fusion of the cervical spine may be life-saving in the prevention of spinal cord compression [66]. Surgical decompression may be required for cord compression. There is a tendency for the prognosis to be better in females. Osteotomies may be useful in correction of the genu valgum [64, 67]. Any surgery should be undertaken with caution because of the risk of atlantoaxial instability and because of the deformity of the chest and its effect on cardiopulmonary function [68]. Hearing aids may be useful [29]. The instability of the wrists, which makes working with the hands very difficult, may be improved by the use of wrist splints.

Enzyme replacement therapy is under exploration in knockout mouse models. In a study of recombinant human (rh)GALNS in MPS IVA fibroblasts, there was dose-dependent uptake via mannose-6-phosphate receptor mechanism and restoration of enzyme activity. In MPS IVA chondrocytes, rhGALNS was taken up and internalized into lysosomes. Enzyme activity previously undetectable became normal and there was decreased storage of keratan sulfate. In wild-type mice, intravenous injection of rhGALNS yielded distribution throughout cardiac valves and the growth plate [69].

Chaperone therapy has been explored in patient fibroblasts; lipid accumulation improved and enzyme protein degradation was inhibited [70].

Gene transfer therapy has been studied by transduction of human MPS IVA fibroblasts using adenoassociated virus (AAV)-based vectors, which carry human *GALNS* cDNA [71]. Activity levels of *GALNS* of 15–54 percent of wild type were observed. In murine MPS IVA chondrocytes, enzyme activities following transduction were up to 70 percent of control. Cotransduction with the sulfatase-modifying factor 1 (SUMF1) gene led to a further four-fold increase in enzyme activity [72]. Improved targeting to bone was obtained in Morquio A mice by bioengineering human *GALNS* to extend the N-terminus. Mice treated from the neonatal period had appreciable reduction of storage in tissues [72].

REFERENCES

1. Morquio L. Sur une form de dystrophie osseuse familiale. *Arch Med Enfants* 1929; 32: 129.

2. Brailsford JF. Chondro-osteo-dystrophy: roentgenographic and clinical features of a child with dislocation of vertebrae. *Am J Surg* 1929; **7**: 404.
3. Maroteaux P, Lamy M. Opacités cornéennes et trouble métabolique dans la maladie de Morquio. *Rev Fr Etud Clin Biol* 1961; **6**: 48.
4. Minami R, Katsuyuki A, Kudoh T *et al*. Identification of keratan sulfate in liver affected by Morquio syndrome. *Clin Chim Acta* 1979; **93**: 207.
5. Gadbois P, Moreau J, Laberge C. La maladie de Morquio dans la province de Québec. *Union Med Can* 1973; **102**: 602.
6. Matalon R, Arbogast B, Justice P *et al*. Morquio's syndrome: deficiency of a chondroitin sulfate N-acetylhexosamine sulfate sulfatase. *Biochem Biophys Res Commun* 1974; **61**: 759.
7. Singh J, Differrante NM, Nieves P, Tavella D. N-Acetylgalactosamine-6-sulfate sulfatase in man: absence of the enzyme in Morquio disease. *Clin Invest* 1976; **57**: 1036.
8. Horwitz AL, Dorfman A. The enzymatic defect in Morquio's disease: the specificity of N-acetylhexosamine sulfatases. *Biochem Biophys Res Commun* 1978; **80**: 819.
9. Tomatsu S, Montaña AM, Oikawa H *et al*. Mucopolysaccharidosis type IVA (Morquio A disease): clinical review and current treatment. *Curr Pharm Biotechnol* 2011; **12**: 931.
10. Arbisser AI, Donnelly KA, Scott CI Jr *et al*. Morquio-like syndrome with beta-galactosidase deficiency and abnormal hexosamine sulfatase activity: mucopolysaccharidosis IV B. *Am J Med Genet* 1977; **1**: 195.
11. O'Brien JS, Gugler E, Giedion A *et al*. Spondyloepiphyseal dysplasia corneal clouding normal intelligence and acid beta-galactosidase deficiency. *Clin Genet* 1976; **9**: 495.
12. Groebe H, Krins M, Schmidberger H *et al*. Morquio syndrome (mucopolysaccharidosis IV B) associated with beta-galactosidase deficiency: a report of two cases. *Am J Hum Genet* 1980; **32**: 258.
13. Tomatsu S, Fukuda S, Masue M *et al*. Morquio disease: isolation characterization and expression of full length DNA for human N-acetylgalactosamine-6-sulfatase. *Biochem Biophys Res Commun* 1991; **181**: 677.
14. Masuno M, Tomatsu S, Nakashima Y *et al*. Mucopolysaccharidosis IV A: assignment of the human N-acetylgalactosamine-6-sulfate sulfatase (GALNS) gene to chromosome 16 q24. *Genomics* 1993; **16**: 777.
15. Baker E, Guo X-H, Orsborn A *et al*. The Morquio A syndrome (mucopolysaccharidosis IVA) gene maps to 16 q243. *Am J Hum Genet* 1993; **52**: 96.
16. Tomatsu S, Fukuda S, Cooper A *et al*. Mucopolysaccharidosis IVA: identification of a common missense mutation I113 F in the N-acetylgalactosamine-6-sulfate sulfatase gene. *Am J Hum Genet* 1995; **57**: 556.
17. Hori T, Tomatsu S, Nakashima Y *et al*. Mucopolysaccharidosis type IVA: common double deletion in the N-acetylgalactosamine-6-sulfatase gene (GALNS). *Genomics* 1995; **26**: 535.
18. Tomatsu S, Fukuda S, Yamagishi A *et al*. Mucopolysaccharidosis IVA: four new exonic mutations in patients with N-acetylgalactosamine-6-sulfate sulfatase deficiency. *Am J Hum Genet* 1996; **58**: 950.
19. Tomatsu S, Fukuda S, Cooper A *et al*. Two new mutations Q473 X and N487 S in a Caucasian patient with mucopolysaccharidosis IVA (Morquio disease). *Hum Mutat* 1995; **6**: 195.
20. Shows TB, Scrafford-Wolff LR, Brown JA, Meisler M. Assignment of a beta-galactosidase level (beta GALA) to chromosome 3 in man. *Cytogenet Cell Genet* 1978; **22**: 219.
21. Suzuki Y, Oshima A. A beta-galactosidase gene mutation identified in both Morquio B disease and infantile G(M1) gangliosidosis (letter). *Hum Genet* 1993; **91**: 407.
22. McKusick VA. The mucopolysaccharidoses. In: McKusick VA (ed.). *Heritable Disorders of Connective Tissue*, 4th edn. St Louis: CV Mosby, 1972: 583.
23. Hecht JT, Scott CI Jr, Smith TK, Williams JC. Mild manifestations of the Morquio syndrome (letter). *Am J Med Genet* 1984; **18**: 369.
24. Beck M, Glossl J, Grubisic A, Spranger J. Heterogeneity of Morquio disease. *Clin Genet* 1986; **29**: 325.
25. Maroteaux P, Stanescu V, Stanescu R *et al*. Heterogeneity des formes frustes de la maladie de Morquio. *Arch Franc Pédiatr* 1982; **39**: 761.
26. Holzgreve W, Grobe H, von Figura K *et al*. Morquio syndrome: clinical findings in 11 patients with MPS IVA and 2 patients with MPS IVB. *Hum Genet* 1981; **57**: 360.
27. Van Noorden GK, Zellweger H, Ponseti IV. Ocular findings in Morquio – Ullrich's disease. *Arch Ophthalmol* 1960; **64**: 585.
28. Cahane M, Treister G, Abraham FA, Melamed S. Glaucoma in siblings with Morquio syndrome. *Br J Ophthalmol* 1990; **74**: 382.
29. Reidner ED, Levin LS. Hearing patterns in Morquio's syndrome (mucopolysaccharidosis IV). *Arch Otolaryngol* 1977; **103**: 518.
30. Northover H, Cowie RA, Wraith JE. Mucopolysaccharidosis type IVA (Morquio syndrome): a clinical review. *J Inher Metab Dis* 1996; **19**: 357.
31. Levin LS, Jorgenson RJ, Salinas CF. Oral findings in the Morquio syndrome (mucopolysaccharidosis IV). *Oral Surg Oral Med Oral Pathol* 1975; **39**: 390.
32. Nelson J, Kinirons M. Clinical findings in 12 patients with MPS IV A (Morquio's disease): further evidence for heterogeneity. Part II: dental findings. *Clin Genet* 1988; **33**: 121.
33. John RM, Hunter D, Swanton RH. Echocardiographic abnormalities in type IV mucopolysaccharidosis. *Arch Dis Child* 1990; **65**: 746.
34. Langer LO Jr, Carey LS. The roentgenographic features of the KS mucopolysaccharidosis of Morquio (Morquio-Brailsford's disease). *Am J Roentgenol Radium Ther Nucl Med* 1966; **97**: 1.
35. Lipson SJ. Dysplasia of the odontoid process in Morquio's syndrome causing quadriplegia. *J Bone Joint Surg* 1977; **59**: 340.
36. Couprie J, Denis P, Guffon N *et al*. Ocular manifestations in patients affected by Morquio syndrome (MPS IV). *J Fr Ophthalmol* 2010; **33**: 617.
37. Blaw ME, Langer LO. Spinal cord compression in Morquio-Brailsford disease. *J Pediatr* 1969; **74**: 593.

38. Hughes DG, Chadderton RD, Cowie RA *et al.* MRI of the brain and craniocervical junction in Morquio's disease. *Neuroradiology* 1997; **39**: 381.
39. Greenberg AD. Atlantoaxial dislocations. *Brain* 1968; **91**: 655.
40. Tojak JE, Ho CH, Roesel RA *et al.* Morquio-like syndrome (MPS IV B) associated with deficiency of beta-galactosidase. *Johns Hopkins Med J* 1980; **146**: 75.
41. Scheie HG, Hambrick GW Jr, Barnes LA. A newly recognized forme fruste of Hurler's disease (gargoylism). *Am J Ophthalmol* 1962; **53**: 753.
42. Danes VS, Grossman H. Bone dysplasias including Morquio's syndrome studied in skin and fibroblast cultures. *Am J Med* 1969; **47**: 708.
43. Koto A, Horwitz AL, Suzuki K *et al.* The Morquio syndrome: neuropathology and biochemistry. *Ann Neurol* 1978; **4**: 26.
44. Applegarth DA, Toone JR, Wilson RD *et al.* Morquio disease presenting as hydrops fetalis and enzyme analysis of chorionic villus tissue in a subsequent pregnancy. *Pediatr Pathol* 1987; **7**: 593.
45. Qubbaj W, Aida I Al Aqeel Al, Al-Hassnan Z *et al.* Preimplantation genetic diagnosis of Morquio disease. *Prenat Diagn* 2008; **28**: 900.
46. Humbel R, Marchal C, Fall M. Diagnosis of Morquio's disease: a simple chromatographic method for the identification of keratosulfate in urine. *J Pediatr* 1972; **81**: 107.
47. Tomatsu S, Okamura K, Taketani T *et al.* Development and testing of new screening method for keratan sulfate in Mucopolysaccharidosis IVA. *Pediatr Res* 2004; **55**: 592.
48. Tomatsu S, Montaña AM, Oguma T *et al.* Validation of keratan sulfate level in mucopolysaccharidosis type IVA by liquid chromatography-tandem mass spectrometry. *J Inherit Metab Dis* 2010; Jan 27. [Epub ahead of print].
49. Fujimoto A, Horwitz AL. Biochemical defect of non-keratan-sulfate-excreting Morquio syndrome. *Am J Med Genet* 1983; **15**: 265.
50. Glossl J, Lembeck K, Gamse G, Kresse H. Morquio's disease type A: absence of material cross-reacting with antibodies against N-acetylgalactosamine-6-sulfate sulfatase. *Hum Genet* 1980; **54**: 87.
51. van der Horst GTJ, Kleijer WJ, Hoogeveen AT *et al.* Morquio B syndrome: a primary defect in beta-galactosidase. *Am J Med Genet* 1983; **16**: 261.
52. Khedhiri S, Chkioua L, Ferchichi S *et al.* Polymorphisms in Tunisian patients with N-acetylgalactosamine-6-sulfate sulfatase gene deficiency: implication in Morquio A disease. *Diagn Pathol* 2011; **20**: 6.
53. Montano AM, Orii KO, Grubb JH *et al.* Spectrums of mutations in mucopolysaccharidosis IVA (Morquio disease) gene. *Proc Jpn Soc Inherit Metab Dis* 1991; **44**: 172.
54. Fukuda S, Tomatsu S, Masue M *et al.* Mucopolysaccharidosis type IVA: N-acetylgalactosamine-6-sulfate sulfatase exonic point mutations in classical Morquio and mild cases. *J Clin Invest* 1992; **90**: 1049.
55. Zhao Y, Meng Y, Guo Y *et al.* Identification of a novel mutation of GALNS gene from a Chinese pedigree with mucopolysaccharidosis type IV A. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2011; **28**: 241.
56. Wang Z, Zhang W, Wang Y *et al.* Mucopolysaccharidosis IVA mutations in Chinese patients: 16 novel mutations. *J Hum Genet* 2010; **55**: 534.
57. Oshima A, Yoshida K, Shimmoto M *et al.* Human beta-galactosidase gene mutations in Morquio B disease. *Am J Hum Genet* 1991; **49**: 1091.
58. Ishii N, Oohira T, Oshima A *et al.* Clinical and molecular analysis of a Japanese boy with Morquio B disease. *Clin Genet* 1995; **48**: 103.
59. Hofer D, Paul K, Fantur K *et al.* GM1 gangliosidosis and Morquio B disease: expression analysis of missense mutations affecting the catalytic site of acid beta-galactosidase. *Hum Mutat* 2009; **30**: 1214.
60. Kresse H, von Figura K, Kelen U *et al.* Enzymatic diagnosis of the genetic mucopolysaccharide storage disorders – an extension. *Meth Enzymol* 1982; **83**: 559.
61. Glossl J, Kresse H. A sensitive procedure for the diagnosis of N-acetylgalactosamine-6-sulfate sulfatase deficiency in classical Morquio's disease. *Clin Chem Acta* 1978; **88**: 111.
62. Khaliq T, Sadilek M, Scott CR *et al.* Tandem mass spectrometry for the direct assay of lysosomal enzymes in dried blood spots: application to screening newborns for mucopolysaccharidosis IVA. *Clin Chem* 2011; **57**: 128.
63. Duffey TA, Khaliq T, Scott CR *et al.* Design and synthesis of substrates for newborn screening of Maroteaux-Lamy and Morquio A syndromes. *Bioorg Med Chem Lett* 2010; **20**: 5994.
64. Norum RA. Nonkeratosulfate-excreting Morquio's syndrome in four members of an inbred group. In: *Skeletal Dysplasias, Clinical Delineation of Birth Defects*, Part IV. New York: The National Foundation – March of Dimes, 1979: 334.
65. Zustin J. Morquio disease: the role of cartilage canals in the pathogenesis of chondrogenic dwarfism. *Med Hypotheses* 2010; **75**: 642.
66. Kopits SE, Perovic MN, McKusick VA *et al.* Congenital atlantoaxial dislocations in various forms of dwarfism. *J Bone Joint Surg* 1972; **54A**: 1349.
67. Kopits SE. Orthopedic complications of dwarfism. *Clin Orthop* 1976; **114**: 153.
68. Jones AEP, Croley TF. Morquio syndrome and anesthesia. *Anesthesiology* 1979; **51**: 261.
69. Dvorak-Ewell M, Wendt D, Hague C *et al.* Enzyme replacement in a human model of mucopolysaccharidosis IVA *in vitro* and its biodistribution in the cartilage of wild type mice. *PLoS One* 2010; **16**: e12194.
70. Higaki K, Li L, Bahrudin U *et al.* Chemical chaperone therapy: chaperone effect on mutant enzyme and cellular pathophysiology in β -galactosidase deficiency. *Hum Mutat* 2011; **21** [Epub ahead of print].
71. Alméciga-Díaz CJ, Montaña AM, Tomatsu S, Barrera LA. Adeno-associated virus gene transfer in Morquio A disease – effect of promoters and sulfatase-modifying factor 1. *FEBS J* 2010; **277**: 3608.
72. Tomatsu S, Montaña AM, Dung VC *et al.* Enhancement of drug delivery: enzyme-replacement therapy for murine Morquio A syndrome. *Molec Ther* 2010; **18**: 1094.

Maroteaux-Lamy disease/mucopolysaccharidosis VI/N-acetylgalactosamine-4-sulfatase deficiency

Introduction	597	Treatment	602
Clinical abnormalities	597	References	602
Genetics and pathogenesis	601		

MAJOR PHENOTYPIC EXPRESSION

Shortness of stature, limitation of joint motion and contractures, corneal clouding, hepatosplenomegaly, dysostosis multiplex, excretion of dermatan sulfate, and deficiency of N-acetylgalactosamine-4-sulfatase (arylsulfatase B).

INTRODUCTION

A distinct mucopolysaccharidosis was first recognized by Maroteaux, Lamy and colleagues [1] in 1963 as a syndrome in which patients displayed some of the features of the Hurler syndrome, but had normal intelligence [1, 2]. Furthermore, the mucopolysaccharide found in the urine was predominantly dermatan sulfate. A number of variants have now been described in which the range of severity is quite broad.

The molecular defect (Figure 81.1) is in the enzyme N-acetylgalactosamine-4-sulfatase [3, 4]. It catalyzes the

removal of sulfate moieties from both dermatan sulfate and chondroitin-4-sulfate. This protein is the Maroteaux-Lamy corrective factor [5]. The human cDNA has been cloned [6] and the gene has been mapped to chromosome 5q13-14 [7]. A variety of mutations have been identified [8, 9].

CLINICAL ABNORMALITIES

The classic patient with the Maroteaux-Lamy syndrome develops impressively short stature [10, 11]. The patient is often first brought to the physician at two to three years of age because of impaired growth. The problem involves both the trunk and the extremities. By this time, the patient may be found to have the deformities and facial characteristics of a mucopolysaccharidosis (Figures 81.2, 81.3, 81.4, 81.5, 81.6, 81.7, 81.8, 81.9, 81.10 and 81.11). The facial features are recognizably coarse (Figures 81.2, 81.3, and 81.6), but they are considerably more subtle than those of the patient with the Hurler syndrome. The breathing may be noisy from early infancy. A large head or prominent chest may be present at birth. There may be umbilical or inguinal hernias, and surgical repair may be required in the first years of life [10].

Ultimate height of 107–138 cm may be reached by six to eight years, the age at which growth usually stops. The head appears larger than the body. The skin may appear tight. Hirsutism is common. There may be macroglossia and protrusion of the tongue. The typical appearance of

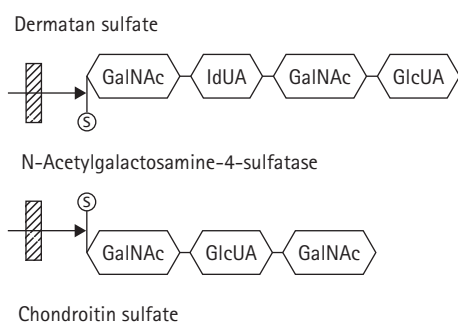


Figure 81.1 Degradation of dermatan sulfate and chondroitin sulfate. N-acetylgalactosamine-4-sulfatase, the site of the defect of Maroteaux-Lamy syndrome, is active in the degradation of both glucosaminoglycans.

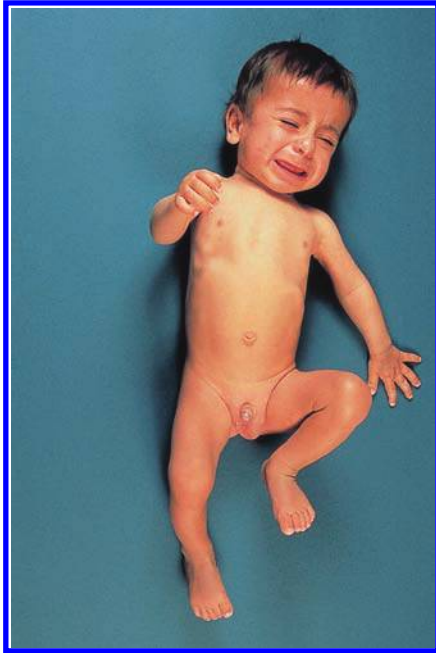


Figure 81.2 TSMG: A one-year-old Saudi Arabian boy with the Maroteaux-Lamy syndrome. He was very short. The facial features were coarse, but much less so than in Hurler syndrome or in most patients with Hunter syndrome.



Figure 81.4 TSMG: In the lateral view, the gibbus is characteristic.



Figure 81.3 A seven-year-old boy with Maroteaux-Lamy disease. Illustrated are the shortness of stature, somewhat coarse facies and genu valgum. He also had cloudy corneas. (Illustration was kindly provided by Dr Philip Benson.)

the child with this disorder is of a short trunk, protuberant abdomen, and lumbar lordosis.

Changes of the joints are progressive, and motion becomes increasingly limited. Genu valgum and a position



Figure 81.5 Close up of the face reveals coarse features, a flattened nasal bridge, and a large tongue.

of semiflexion of the knees are characteristic, giving the child a crouched stance. A claw-hand deformity develops that is typical of mucopolysaccharidosis (Figure 81.9). There may be flexion contractures of the fingers, as well as the knees and elbows. The differential diagnosis may suggest mucopolidosis III (Chapter 84). A carpal tunnel



Figure 81.6 TSMG: At 18 months. The features were increasingly coarse.



Figure 81.8 HAH: Close up of the face illustrates the low hair line, flat, coarse facies, and macroglossia. He had bilateral corneal clouding.

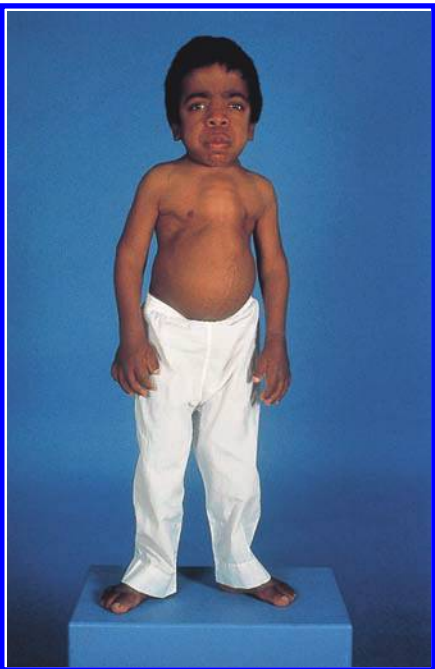


Figure 81.7 HAH: A nine-year-old Saudi boy with Maroteaux-Lamy disease. Facial features were coarse. He was very short. The activity of arylsulfatase B in fibroblasts was 4 percent of control.

syndrome may contribute to the limitation of hand motion. The subcutaneous tissues of the volar surfaces of the second to fourth fingers may be thickened, as in Dupuytren contractures [10]. Lumbar kyphosis and anterior protrusion of the sternum are also progressive.

Hepatomegaly is regularly observed in patients over six



Figure 81.9 TSMG: At 18 months. The hand was typical of a mucopolysaccharidosis with broadening of the digits and claw-shaped contractures.

years of age, and splenomegaly is found in about half of the patients. Some patients have had frequent episodes of diarrhea.

Cardiac abnormality is an important component of this syndrome [12]. Murmurs heard indicate valvular involvement. The mitral and aortic valves become thickened, calcified, and stenotic [13, 14]. A murmur of aortic stenosis is frequently present [15–17], and mitral or aortic regurgitation may also be present. There may be right as well as left ventricular failure. An unusual presentation is with acute infantile cardiomyopathy [18, 19]. Cardiac



Figure 81.10 HAH: At nine years; the claw hand deformity was very prominent.



Figure 81.11 HAH: The foot was also broad and the toes particularly wide.

failure may be the cause of death, which usually occurs before 30 years of age in the classic form of the disease. Some have died of pulmonary infection. Another pulmonary complication is obstructive sleep apnea.

The corneas develop opacities at an early stage that are detectable by slit lamp examination and progressively become clinically cloudy. This is especially dense at the periphery and it may lead to visual impairment. Glaucoma was reported in four adult women [20]. Deafness is a regular feature, related at least in part to recurrent otitis media.

In contrast to most of the mucopolysaccharidoses, the Maroteaux-Lamy disease is characterized by normal intelligence. Two families have been reported in which there was impaired mental development, but this may have had some other etiology [21, 22].

On the other hand, neurologic complications occur frequently [23–26]. Hydrocephalus may result from pachymeningeal thickening. Ventricular shunting may be required. Myelopathy may result from cord compression following atlantoaxial subluxation. The dura may also

be thickened in the cervical region, leading to insidious compression of the cord. The end result is spastic paraplegia [27]. Myelopathy due to compression may also result from developmental abnormalities of the vertebral bodies and kyphoscoliosis [28]. Papilledema and progressive loss of vision may be a consequence of increased intracranial pressure [11]. Neurologic deterioration has been observed during pregnancy [24]. A complete lack of development of secondary sexual characteristics, as well as an unusual degree of dwarfism in a patient, suggested that the anterior pituitary was also affected.

Patients with milder variants may present with hip dysplasia resembling Legg-Perthes disease. Some present first as adults with disease of the hips [29].



Figure 81.12 TSMG: Lateral roentgenogram of the spine. The vertebral body of L1 was hypoplastic and prominent anteriorly. The gibbus deformity was in this area.



Figure 81.13 TSMG: Roentgenogram of the arm. The bones are all thickened and poorly modeled.

Roentgenograms demonstrate the typical findings of dysostosis multiplex (Figures 81.12, 81.13, and 81.14) [11]. Roentgenograms of the hand may in classic examples be indistinguishable from that of Hurler disease (Chapter 76). In some patients diaphyseal constriction may suggest Morquio disease (Chapter 80). The epiphyses are abnormal. Femoral heads may be particularly dysplastic, and coxa valga is a regular occurrence. The iliac bodies tend to be small and constricted, and the wings flare. The acetabula are small and hypoplastic and the acetabular roofs are oblique. Ossification of the femoral head may be irregular, and this is the reason patients have been thought to have Legg-Perthes disease. The femoral necks may turn outward. Widening of the epiphyseal plates may resemble metaphyseal chondrodysplasia.

The long bones are short and thickened or distended (Figures 81.13) and the radius and ulna may be bowed. In addition, there may be a localized metaphyseal constriction. This may be particularly striking in the surgical neck of the femur. The ribs may be abnormally broad and short, but narrowed at the vertebral ends so that they resemble canoe paddles [11]. There may be oval radiolucencies in the tibial and distal femoral metaphyses, representing residual islands of cartilage. The first lumbar vertebra may be cuneiform. Beaking or anterior hypoplasia occurs typically in this vertebra and in T12; their posterior displacement causes a gibbus deformity. The odontoid may be quite hypoplastic. Along with macrocephaly, the large sella turcica may be omega or J-shaped (Figure 81.14). The calvaria may have a ground-glass appearance, and the mastoids may be sclerotic. Eruption of the teeth may be impaired [30, 31].



Figure 81.14 TSMG: Roentgenogram of the skull. The cranial vault was high and the sella J-shaped. Adenoid tissue was prominent.

The height of the mandible may be reduced, and teeth may be displaced so far toward its inferior border that the cortex of the mandible is nearly penetrated by the roots of the teeth.

Cytoplasmic inclusions are more prominent in the Maroteaux-Lamy syndrome than in any of the other mucopolysaccharidoses. They can be seen in 90–100 percent of granulocytes (Alder granules) [32] and as many as 50 percent of lymphocytes [10]. The inclusions are metachromatic. Lysosomal inclusions are also seen in Kupffer cells and in hepatocytes [33], as well as in platelets [34], and in cells of the conjunctiva, cornea, and skin [35]. Fibroblasts may contain large, clear, juxtanuclear inclusions. Large quantities of dermatan sulfate are excreted in the urine, but the total mucopolysaccharide in the urine may be normal [30]; so screening tests for urinary mucopolysaccharidosis may be misleading.

GENETICS AND PATHOGENESIS

The inheritance of this disorder is autosomal recessive. Multiple affected siblings and normal parents have been observed in a number of families, and consanguinity has been documented [10, 36]. The site of the defect is in the activity of N-acetylgalactosamine-4-sulfatase (Figure 81.1), which is coded for by a gene on chromosome 5 at position q13-14 [6].

N-acetylgalactosamine-4-sulfatase catalyzes the hydrolysis of the sulfate from moieties of N-acetylgalactosamine-4-sulfate which occur in dermatan sulfate. The moieties are also found in chondroitin-4-sulfate. Defective activity in this hydrolysis may account for some of the abnormalities in the joints, but chondroitin sulfate is not found in the urine because it can be degraded by hyaluronidase. The enzyme is also known as arylsulfatase B. The human enzyme has been purified [37, 38]. Its biosynthesis and processing involve the phosphorylation of mannose moieties and proteolysis in the classic lysosomal enzyme pattern [39, 40].

It was initially demonstrated, using artificial substrate, that this enzyme was deficient in Maroteaux-Lamy disease [6, 41–44]. The enzyme also has uridine diphospho-N-acetylgalactosamine-4-sulfate sulfohydrolyase activity [44].

cDNAs for the human [4, 5] and feline [45] enzymes have been cloned. The human monomeric protein contains 533 amino acids, including a 46 amino acid signal peptide. There is considerable homology with other sulfatases.

Correlation of clinical severity with the amount of residual enzyme activity has not been possible, but over 20 percent of normal activity is consistent with a normal phenotype [46]. The disorder has been diagnosed prenatally by enzyme assay [47]. Heterozygosity may also be demonstrated by assay of arylsulfatase B activity [48].

Analysis of the nature of mutation promises to permit better correlation of genotype with phenotype. Southern blot analysis of genomic DNA of 17 patients revealed no

deletions or rearrangements [49]. A majority of patients were compound heterozygotes for two mutations. Homozygous deletion of a base, DG238, was found in a patient with a severe disease [9]. Severe disease was also present in a patient with different deletions on the two alleles [50]. A similar degree of severity was found in a patient with a missense conversion of cystine 117 to arginine [8], while the more conservative conversion of glycine 137 to valine was found in a patient with an intermediate phenotype [51]. Two different frameshift mutations led to stop codons in a child with severe disease [50]. Mild disease was found in a patient with two mutant alleles, one a leucine 266 to proline change, and the other a cysteine 405 to tyrosine [7].

TREATMENT

Surgical corrective procedures may be useful in the management of carpal tunnel syndrome, the hips, or the cornea. Laminectomy and removal of the thickened dura has led to improvement in myelopathy [27]. Replacement of aortic and mitral valves has been successful [14].

Bone marrow transplantation was carried out in a 13-year-old girl with normal intelligence who had advanced cardiac failure and obstructive apnea, requiring oxygen during sleep and a tracheotomy [52]. Following successful engraftment, urinary excretion of mucopolysaccharide decreased, as did hepatosplenomegaly. Cardiopulmonary function became normal. Visual acuity improved, though the cloudy appearance of the cornea was unchanged. There was no obvious change in the dysostosis multiplex. Bone marrow transplantation has also been carried out in a feline model of the syndrome in which arylsulfatase B activity was deficient [53–55]. Corneal clouding of these animals disappeared. A model in rats has also been established [56] which should facilitate experimental therapy. Needle biopsy of the liver in human transplanted patients has revealed clearing of Kupffer cells and hepatocytes of stored glycosaminoglycan [57].

REFERENCES

1. Maroteaux P, Leveque B, Maruie J, Lamy M. Une nouvelle dysostose avec élimination urinaire de chondroïtine sulfate B. *Presse Med* 1963; **71**: 1849.
2. Maroteaux P, Lamy M. Hurler's disease Morquio's disease and related mucopolysaccharidoses. *J Pediatr* 1965; **67**: 312.
3. Matalon R, Arbogast B, Dorfman A. Deficiency of chondroitin sulphate N-acetylgalactosamine 4-sulfate sulfatase in Maroteaux-Lamy syndrome. *Biochem Biophys Res Commun* 1974; **61**: 1450.
4. O'Brien JS, Cantz M, Spranger J. Maroteaux-Lamy disease (mucopolysaccharidosis VI) subtype A: deficiency of N-acetylgalactosamine-4-sulfatase. *Biochem Biophys Res Commun* 1974; **60**: 1170.
5. Barton RW, Neufeld EF. A distinct biochemical deficit in the Maroteaux-Lamy syndrome (mucopolysaccharidosis VI). *J Pediatr* 1972; **80**: 114.
6. Schuchman EH, Jackson CE, Desnick RJ. Human arylsulfatase B: MOPAC cloning nucleotide sequence of a full-length cDNA and regions of amino acid identity with arylsulfatases A and C. *Genomics* 1990; **6**: 149.
7. Litjens T, Baker EG, Beckmann KR *et al*. Chromosomal localization of ARSB the gene for human N-acetylgalactosamine-4-sulphatase. *Hum Genet* 1989; **82**: 67.
8. Jin WD, Jackson CE, Desnick RJ, Schuchman EH. Mucopolysaccharidosis type VI: identification of three mutations in the arylsulfatase B gene of patients with the severe and mild phenotypes provides molecular evidence for genetic heterogeneity. *Am J Hum Genet* 1992; **50**: 795.
9. Litjens T, Morris CP, Robertson EF *et al*. An N-acetylgalactosamine-4-sulfatase mutation (DG238) results in a severe Maroteaux-Lamy phenotype. *Hum Mutat* 1992; **1**: 397.
10. Spranger JW, Koch F, McKusick VA *et al*. Mucopolysaccharidosis VI (Maroteaux-Lamy's disease). *Helv Paediatr Acta* 1970; **25**: 337.
11. McKusick VA. *Heritable Disorders of Connective Tissue*. St Louis: CV Mosby, 1972: 611.
12. Krovetz LJ, Schiebler GL. Cardiovascular manifestations of the genetic mucopolysaccharidoses. In: Bergsma D (ed.). *Clinical Delineation of Birth Defects*, XIII Cardiovascular system. Baltimore: Williams and Wilkins, 1972.
13. Tan CT, Schaff HV, Miller FA Jr *et al*. Valvular heart disease in four patients with Maroteaux-Lamy syndrome. *Circulation* 1992; **85**: 188.
14. Marwick TH, Bastian B, Hughes CF, Bailey BP. Mitral stenosis in the Maroteaux-Lamy syndrome: a treatable cause of dyspnea. *Postgrad Med J* 1992; **68**: 287.
15. Di Ferrante N, Hyman BH, Klish W *et al*. Mucopolysaccharidosis VI (Maroteaux-Lamy disease): clinical and biochemical study of a mild variant case. *Johns Hopkins Med J* 1974; **135**: 42.
16. Gloor GA, Tanaka KR, Turner JA, Liu CK. Mucopolysaccharidosis an unusual case of cardiac valvular disease. *Am J Cardiol* 1968; **22**: 133.
17. Wilson CS, Mankin HT, Pluth JR. Aortic stenosis and mucopolysaccharidosis. *Ann Intern Med* 1980; **92**: 496.
18. Miller G, Partridge A. Mucopolysaccharidosis type VI presenting in infancy with endocardial fibroelastosis and heart failure. *Pediatr Cardiol* 1983; **4**: 61.
19. Hayflick S, Rowe S, Kavanaugh-McHugh A *et al*. Acute infantile cardiomyopathy as a presenting feature of mucopolysaccharidosis VI. *J Pediatr* 1992; **120**: 269.
20. Cantor LB, Disseler JA, Wilson FM II. Glaucoma in the Maroteaux-Lamy syndrome. *Am J Ophthalmol* 1989; **108**: 426.
21. Taylor HR, Hollows FC, Hopwood JJ, Robertson EF. Report of mucopolysaccharidosis occurring in Australian aborigines. *J Med Genet* 1978; **15**: 455.
22. Vestermark S, Tonnesen T, Andersen MS, Guttler F. Mental retardation in a patient with Maroteaux-Lamy. *Clin Genet* 1987; **31**: 114.

23. Goldberg MF, Scott CI, McKusick VA. Hydrocephalus and papilledema in the Maroteaux-Lamy syndrome (mucopolysaccharidosis type VI). *Am J Ophthalmol* 1970; **69**: 969.
24. Sostrin RD, Hasso AN, Peterson DI, Thompson JR. Myelographic features of mucopolysaccharidosis: a new sign. *Radiology* 1977; **125**: 421.
25. Peterson DI, Bucchus A, Seaich L, Kelly TE. Myelopathy associated with Maroteaux-Lamy syndrome. *Arch Neurol* 1975; **32**: 127.
26. Upton ARM, McComas AJ. The double crush in nerve-entrapment syndromes. *Lancet* 1973; **2**: 359.
27. Young R, Kleinman G, Ojemann RG *et al.* Compressive myelopathy in Maroteaux-Lamy syndrome: clinical and pathological findings. *Ann Neurol* 1980; **8**: 336.
28. Wald SL, Schmidek HH. Compressive myelopathy associated with type VI mucopolysaccharidosis (Maroteaux-Lamy syndrome). *Neurosurgery* 1984; **14**: 83.
29. Tonnesen T, Gregersen HN, Guttler F. Normal MPS excretion but dermatan sulphaturia combined with a mild Maroteaux-Lamy phenotype. *J Med Genet* 1991; **28**: 499.
30. Grossman H, Dorst JP. The mucopolysaccharidoses. In: Kaufman H (ed.). *Progress in Pediatric Radiology*, vol. IV. Chicago: Year Book Medical, 1972: 495.
31. Worth HM. The Hurler's syndrome; a study of radiologic appearances in the jaws. *Oral Surg* 1966; **22**: 21.
32. Alder A. Ueber konstitutionell bedingte Granulationsveraenderungen der Leukocyten. *Dtsch Arch Klin Med* 1939; **183**: 372.
33. Tondeur M, Neufeld EF. The mucopolysaccharidoses: biochemistry and ultrastructure. In: Good RA, Day SB, Yunis JJ (eds). *Molecular Pathology*. Springfield: Charles C Thomas, 1975: 600.
34. Levy LA, Lewis JC, Sumner TE. Ultrastructures of Reilly bodies (metachromatic granules) in Maroteaux-Lamy syndrome (mucopolysaccharidosis VI): a histochemical study. *Am J Clin Pathol* 1980; **73**: 416.
35. Quigley HA, Kenyon KR. Ultrastructural and histochemical studies of a newly recognized form of systemic mucopolysaccharidosis (Maroteaux-Lamy syndrome mild phenotype). *Am J Ophthalmol* 1974; **77**: 809.
36. Slot G, Burgess GL. Gargoylism. *Proc R Soc Med* 1937; **31**: 1113.
37. McGovern MM, Vine DT, Haskins ME, Desnick RJ. Purification and properties of feline and human arylsulfatase B isozymes: evidence for feline homodimeric and human monomeric structures. *J Biol Chem* 1982; **257**: 12605.
38. Gibson GJ, Saccone GTP, Brooks DA *et al.* Human N-acetylgalactosamine-4-sulphate sulphatase: purification monoclonal antibody production and native and subunit M values. *Biochem J* 1987; **248**: 755.
39. Steckel F, Hasilik A, von Figura K. Biosynthesis and maturation of arylsulfatase B in normal and mutant cultured human fibroblasts. *J Biol Chem* 1983; **258**: 14322.
40. Taylor JA, Gibson GJ, Brooks DA, Hopwood JJ. Human N-acetylgalactosamine-4-sulphatase biosynthesis and maturation in normal Maroteaux-Lamy and multiple-sulphatase-deficiency fibroblasts. *Biochem J* 1990; **268**: 379.
41. Fluharty AL, Stevens RL, Sander DL, Kihara H. Arylsulfatase B deficiency in Maroteaux-Lamy syndrome cultured fibroblasts. *Biochem Biophys Res Commun* 1974; **59**: 455.
42. Shapira E, De Gregorio RP, Matalon R, Nadler HL. Reduced arylsulfatase B activity of the mutant enzyme protein in Maroteaux-Lamy syndrome. *Biochem Biophys Res Commun* 1975; **62**: 448.
43. Stumpf DA, Austin JH, Crocker AC, Lafrance M. Mucopolysaccharidosis Type VI (Maroteaux-Lamy syndrome): arylsulfatase B deficiency in tissues. *Am J Dis Child* 1973; **126**: 747.
44. Fluharty AL, Stevens RL, Fung D *et al.* Uridine diphospho-N-acetylgalactosamine-4-sulfate sulfohydrolase activity of human arylsulfatase B and its deficiency in the Maroteaux-Lamy syndrome. *Biochem Biophys Res Commun* 1975; **64**: 955.
45. Jackson CE, Yuhki N, Desnick RJ *et al.* Feline arylsulfatase B (ARSB): isolation and expression of the full length cDNA sequence comparison with human ARSB and gene localization to feline chromosome A1. *Genomics* 1992; **14**: 403.
46. Brooks DA, McCourt PAG, Gibson GJ *et al.* Analysis of N-acetylgalactosamine-4-sulfatase protein and kinetics in mucopolysaccharidosis type VI patients. *Am J Hum Genet* 1991; **48**: 710.
47. Van Dyke DL, Fluharty AL, Schafer IA *et al.* Prenatal diagnosis of Maroteaux-Lamy syndrome. *Am J Med Genet* 1981; **8**: 235.
48. Beratis NG, Turner BM, Weiss R, Hirschhorn K. Arylsulfatase B deficiency in Maroteaux-Lamy syndrome: cellular studies and carrier identification. *Pediatr Res* 1975; **9**: 475.
49. Litjens T, Brooks DA, Peters C *et al.* Identification expression and biochemical characterization of N-acetylgalactosamine-4-sulfatase mutations and relationship with clinical phenotype in MPS-VI patients. *Am J Hum Genet* 1996; **58**: 1127.
50. Isbrandt D, Hopwood JJ, von Figura K, Peters C. Two novel frameshift mutations causing premature stop codons in a patient with the severe form of Maroteaux-Lamy syndrome. *Hum Mutat* 1996; **7**: 361.
51. Wicker G, Prill V, Brooks D *et al.* Mucopolysaccharidosis VI (Maroteaux-Lamy syndrome). An intermediate clinical phenotype caused by substitution of valine for glycine at position 137 of arylsulfatase B. *J Biol Chem* 1991; **266**: 27386.
52. Krivit W, Pierpont ME, Ayaz K *et al.* Bone-marrow transplantation in the Maroteaux-Lamy syndrome (mucopolysaccharidosis type VI). Biochemical and clinical status 24 months after transplantation. *N Engl J Med* 1984; **311**: 1606.
53. Wenger D, Casper PW, Thrall MA *et al.* Bone marrow transplantation in the feline model of arylsulfatase B deficiency. In: Krivit W, Paul NW (eds). *Bone Marrow Transplantation for Lysosomal Storage Diseases*. New York: March of Dimes, Birth Defects Foundation, Original Article Series, 1986, 22, 177.
54. Jezyk PF, Haskins ME, Patterson DF *et al.* Mucopolysaccharidosis in a cat with arylsulfatase B deficiency: a model of Maroteaux-Lamy syndrome. *Science* 1977; **198**: 834.
55. Haskins ME, Jezyk PF, Patterson DF. Mucopolysaccharide storage disease in three families of cats with arylsulfatase B

- deficiency: leukocyte studies and carrier identification. *Pediatr Res* 1979; **13**: 1203.
56. Yoshida M, Noguchi J, Ikada H *et al.* Arylsulfatase B-deficient mucopolysaccharidosis in rats. *J Clin Invest* 1993; **91**: 1099.
57. Resnick JM, Krivit W, Snover DC *et al.* Pathology of the liver in mucopolysaccharidosis: light and electron microscopic assessment before and after bone marrow transplantation. *Bone Marrow Transplant* 1992; **10**: 273.

Sly disease/ β -glucuronidase deficiency/ mucopolysaccharidosis VII

Introduction	605	Treatment	608
Clinical abnormalities	606	References	608
Genetics and pathogenesis	607		

MAJOR PHENOTYPIC EXPRESSION

Short stature, coarse facies; hepatosplenomegaly; kyphoscoliosis and vertebral anomalies, including odontoid hypoplasia; impaired mental development; dystosis multiplex; increased excretion of glycosaminoglycans; and deficiency of β -glucuronidase.

INTRODUCTION

In 1973, Sly and colleagues [1] reported a patient with what they recognized as a distinct mucopolysaccharidosis in whom the activity of lysosomal β -glucuronidase was deficient. Complementation studies by Quinton and colleagues [2] on fibroblasts derived from the patient had

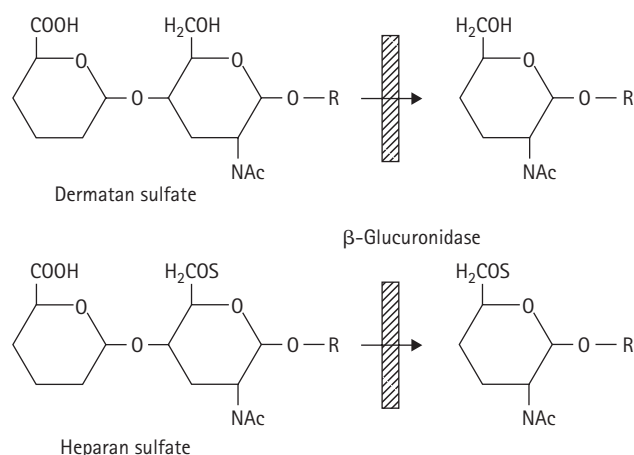


Figure 82.1 The β -glucuronidase reaction. In addition to the removal of glucuronic acid residues from dermatan sulfate and heparan sulfate, the enzyme catalyzes this reaction with the chondroitin sulfates.

revealed this disease to be different from any previously encountered mucopolysaccharidosis. Subsequently, a small number of patients has been described. A considerable variation in clinical expression has been observed [3]. The classic infantile form is similar to Hurler disease, but much milder presentations occur. At the extreme end of the spectrum is the acute fetal or neonatal form characterized by hydrops fetalis [4].

Mucopolysacchariduria in this condition may be mild. The defective enzyme (Figure 82.1), β -glucuronidase [1], catalyzes the removal of glucuronic acid residues that occur in dermatan sulfate and heparan sulfate; it is also active against chondroitin 4- or 6-sulfates, but these compounds are not stored or excreted in the urine, because of the activity of hyaluronidase [5].

β -Glucuronidase was a key enzyme in the development of current understandings of lysosomal enzyme processing. It was in studies of this enzyme that the mannose-6-phosphate recognition marker was first identified [6]. Full-length cDNAs from the human and rodent genes have been cloned, sequenced, and expressed; they encode polypeptides of 651 and 648 amino acids, respectively [7, 8]. The gene is 21 kb in length and contains 12 exons [9]. It is located at chromosome 7 q21-22 [10]. The mutation has been defined in the initial patient [11] and a small number of others [11-13].

CLINICAL ABNORMALITIES

The original patient [1] was characterized by shortness of stature, relatively severe skeletal abnormalities as compared with other mucopolysaccharidoses, and relatively mild impairment of cognitive function. He was first seen at 7 weeks for metatarsus adductus and recognized as having unusual facial features. The nasal bridge was depressed, the nostrils were anteverted, the maxillae prominent, and the eyes were wide and had epicanthal folds. The abdomen was protuberant and the liver palpable 4 cm below the costal margin. The spleen was at 3 cm. There was a long diastasis recti and an umbilical hernia. There was puffy skin over the dorsa of the hands and feet. A thoracolumbar gibbus had already developed. Short stature had been evident at 18 months, and the head circumference reached the 98th percentile by five months. The gibbus increased, and he developed a pigeon breast with a sharp angle between the body of the sternum pointing forward and the xiphoid pointing backward. He developed bilateral inguinal hernias. Hepatomegaly increased.

Developmental milestones and neurologic examination were normal for two years. By three years, impaired development, especially in speech, was evident, but it appeared to be nonprogressive. Orthopedic problems progressed and walking became painful. He died suddenly at 20 years of age, but autopsy did not reveal the cause – a relatively common occurrence in patients with odontoid hypoplasia and other problems about the neck.



Figure 82.2 TM: A boy with mucopolysaccharidosis VII had the classic phenotype. Facial features were coarse. He had hepatosplenomegaly, a gibbus and bilateral inguinal hernia, and developed hydrocephalus [16]. (This illustration was kindly provided by Dr Kenneth Lyons Jones of UCSD.)



Figure 82.3 TM: With time the features were coarser. There was marked gingival hyperplasia. He died at 15 years of age. (This illustration was kindly provided by Dr Kenneth Lyons Jones of UCSD.)

This classic presentation [1, 14–19] of a moderately severe Hurler-like mucopolysaccharidosis with modest impaired mental development (Figures 82.2 and 82.3) represents a relatively uncommon intermediate presentation of MPS VII. There are appreciably milder forms, and the most severe prenatal or neonatal forms appear to be the most common. Clouding of the cornea became evident in the index patient by eight years of age, but in others it has been evident earlier. It can usually be readily demonstrated by slit lamp examination.

Most patients have had frequent upper respiratory infections, and pneumonia has occurred in some. Hernias, shortness of stature [1], relative macrocephaly, and coarse features are regularly observed. Most have had gingival hyperplasia. Gibbus deformity has regularly been reported.

Joint contractures have been observed and also hydrocephalus [16], concomitants of a classic mucopolysaccharidosis. Some have had dislocated hips [18]. Camptodactyly has been noted at birth along with absence of distal phalangeal creases, indicating prenatal onset. All have had hepatosplenomegaly. Developmental delay has been mild to moderate.

A severe example of this phenotype [3] died at 2½ years after a course characterized by marked inhibition of growth, hepatosplenomegaly of neonatal onset, corneal clouding, by seven months, and gingival hypertrophy. Icterus, recurrent diarrhea and hypoalbuminemia may have been unrelated consequences of giant cell hepatitis and carbohydrate intolerance.



Figure 82.4 WM: A 6-week-old infant with Sly disease. He had fetal and neonatal ascites evident at birth and macrocephaly. There was prominent subcutaneous tissue visible in the nares. Liver and spleen were enlarged, and he had bilateral inguinal hernias. He had a gibbus and a prominent manubrium.

The most severe phenotype, a neonatal or fetal form [4, 20–27] is typified by nonimmune hydrops fetalis (Figure 82.4). The metabolic differential diagnosis of hydrops fetus is shown in the Appendix. Three reports were of fetal death, and family histories of hydropic or neonatal patients indicate an increase in spontaneous abortions [26]. This is a distinct presentation for a mucopolysaccharidosis recognizable *in utero* or at least at birth. Dystosis multiplex is present at birth in these patients. The facies is coarse. There is pitting neonatal edema, ascites, and hepatosplenomegaly. Talipes equinovarus has been reported and congenital dislocation of the hip [4]. Cardiomyopathy may be progressive [4]. Death may occur in the first six months.

A number of patients have also been reported with milder manifestations with onset after four years of age or much later and with skeletal manifestations predominating [5, 17, 26, 28–30]. One was 14 years old at the time of report [3] and was well except for hypertension and fibromuscular dysplasia causing narrowing of the aorta and femoral arteries. Height was normal, and dysostosis multiplex of ribs and spine was very mild. Speech therapy was required at three years. Another patient [28] appeared normal at 11 years, except for bilateral club feet, which had been surgically corrected, and frequent upper respiratory infections in childhood. Intelligence was tested as normal. A 13-year-old girl [29] had normal height, moderately impaired mental development, a short neck and protruding sternum, corneal clouding, dysplastic hips, and vertebral abnormalities. There was no hepatosplenomegaly. Another

variant was described [30] as an oligosymptomatic 20-year-old male despite severe skeletal dysplasia. It is clear from these observations that there is a very wide spectrum of clinical phenotypes.

Dysostosis multiplex (Chapter 76) has been present in roentgenograms of patients with β -glucuronidase deficiency, especially those with the classic and neonatal forms. The skull is large and the sella J-shaped. The ribs are spatulate [1, 3, 16]. Vertebrae are shortened and anteriorly beaked, and there may be odontoid hypoplasia [1, 3, 16]. Dysplasia of the hips is associated with hypoplastic ilia [1, 3, 16, 18]. Proximal metacarpals are pointed [1, 16].

Coarse lamellar Alder-Reilly inclusions are seen in peripheral granulocytes [1, 30, 31] and also in the bone marrow. Pathological examination has revealed vacuolated hepatocytes; electron microscopy has shown cytoplasmic membrane-bound vesicles [18]. The stored material stains with alcian blue, and this staining may be seen in cultured fibroblasts, which also display metachromasia.

Glycosaminoglycan excretion is usually moderately increased in this condition [1, 3, 18], but screening tests for mucopolysaccharide excretion may be normal [16], and some adult patients have not had increased glycosaminoglycan excretion. The material has been shown to consist of dermatan sulfate and heparan sulfate [3, 18].

GENETICS AND PATHOGENESIS

The disorder is transmitted in an autosomal recessive fashion via mutant genes on chromosome 7 [10]. Its incidence has been estimated at one in 300,000 live births in British Columbia [32]. The molecular defect is in the enzyme β -glucuronidase. (EC 3.2.1.31) [1, 33]. Cultured fibroblasts from patients accumulate sulfated mucopolysaccharide when incubated with $^{35}\text{SO}_4$, and this abnormality is corrected by the addition of bovine liver β -glucuronidase to the medium [33]. Identity of the corrective factor and the glucuronidase was demonstrated by coelectrophoresis in polyacrylamide gel. Virtually complete deficiency has been demonstrated with a variety of synthetic substrates in leukocytes and in fibroblasts [33]. It has also been detected in serum [18]. The enzyme is a tetramer of 75 kDa subunits [34]. It is synthesized as a precursor protein and processed at the carboxyl end by the loss of the signal peptide [33]. Immunochemical studies have indicated the presence of cross-reacting material (CRM) in patients with the disease [35]. The measurement of enzyme activity has not correlated well with the degree of severity of phenotype.

Reduced levels of enzyme were found in the leukocytes of parents [1]. Prenatal diagnosis is available by the assay of cultured amniocytes or chorionic villus material. In families in which the mutation is known, this is the method of choice for prenatal diagnosis and for carrier detection.

The human and murine gene has considerable homology in the coding region for the mature protein.

Alternate splicing of the human gene leads to two types of cDNA [9], the shorter one containing a large deletion in exon 6. A pseudodeficiency allele was defined in the study of a pseudodeficient mother of a child who carried a mutation, L176 F [36]. The mother had greatly reduced levels of β -glucuronidase without evident clinical effect and a substitution of asparagine for aspartic acid, D152N. The existence of pseudodeficiency greatly complicates prenatal diagnosis by enzyme assay, and also heterozygote detection [37].

In the index patient, there was a compound of two alleles, a missense tryptophan 627 to cysteine and a nonsense arginine 256 to stop [11]. In two Japanese patients, mutations at two CpG sites have been identified: alanine 619 to valine [12] and arginine 382 to cysteine [13]. Four more mutations described in two Caucasian patients [38] were a 38 bp deletion at positions 1642–1679 in exon 10 caused by a single base change that generated a new splice site; and three point mutations – proline 148 to serine, tyrosine to cysteine, and tryptophan 507 to a stop-codon. A prenatally diagnosed patient with hydrops fetalis [39] was found [13] to have a C to T transition that led to a substitution of cysteine for arginine 382. In studies of 21 patients with hydrops fetalis or early severe disease [40], 19 different mutations were reported.

TREATMENT

Specific treatment such as bone marrow transplantation (BMT) has not been reported in humans, but success has been obtained in neonatal mice, as contrasted with adult mice with β -glucuronidase deficiency [41]. Enzyme replacement initiated at birth followed by BMT at 5 weeks was highly successful in this model [42]. This deficiency has also been found in a dog model [42], which has been reported to more closely mimic the human clinical disease and enzyme deficiency than the mouse, in which activity is 20 percent of control. The availability of animals should be useful for the development of gene transfer. Among approaches to gene therapy, affected murine fibroblasts were transfected with a retroviral vector containing human β -glucuronidase cDNA and implanted into mice; there was expression of enzyme *in vivo* and disappearance of lysosomal storage in the liver and spleen [43].

Supportive treatment should include attention to potential cervical instability – for example, during anesthesia. Corneal transplantation may be useful in older patients in whom vision is impaired. Physiotherapy is useful for joint stiffness and the preservation of function.

REFERENCES

1. Sly WS, Quinton BA, McAlister WH, Rimoin DL. β -glucuronidase deficiency: report of clinical radiologic and biochemical features of a new mucopolysaccharidosis. *J Pediatr* 1973; **82**: 249.
2. Quinton BA, Sly WS, McAlister WH *et al.* β -Glucuronidase Deficiency: A New Mucopolysaccharide Storage Disease. Atlantic City, NJ: Society for Pediatric Research, 1971: 198 (Abstr.).
3. Beaudet AL, DiFerrante NM, Ferry GD *et al.* Variation in the pheno-typic expression of β -glucuronidase deficiency. *J Pediatr* 1975; **86**: 388.
4. Nelson A, Peterson L, Frampton B, Sly WS. Mucopolysaccharidosis VII β -glucuronidase deficiency presenting as nonimmune hydrops fetalis. *J Pediatr* 1982; **101**: 574.
5. Lee JES, Falk RE, Ng WG, Donnel GN. β -Glucuronidase deficiency: a heterogeneous mucopolysaccharidosis. *Am J Dis Child* 1985; **139**: 57.
6. Kaplan A, Achord DT, Sly WS. Phosphohexosyl components of a lysosomal enzyme are recognized by pinocytosis receptors on human fibroblasts. *Proc Natl Acad Sci USA* 1977; **74**: 2026.
7. Oshima A, Kyle JW, Miller RD *et al.* Cloning sequencing and expression of cDNA for human glucuronidase. *Proc Natl Acad Sci USA* 1987; **84**: 685.
8. Nishimura Y, Rosenfeld MG, Kreibich G *et al.* Nucleotide sequence of rat preputial β -glucuronidase cDNA and *in vivo* insertion of its encoded polypeptide in microsomal membranes. *Proc Natl Acad Sci USA* 1986; **83**: 7292.
9. Miller RD, Hoffmann JW, Powell PP *et al.* Cloning and characterization of the human β -glucuronidase gene. *Genomics* 1990; **7**: 280.
10. Speleman F, Vervoort R, Van Roy N *et al.* Localization by fluorescence *in situ* hybridization of the human functional beta-glucuronidase gene (GUSB) to 7 q1121-q1122 and two pseudogenes to 5 p13 and 5 q13. *Cytogenet Cell Genet* 1996; **72**: 53.
11. Shipley JM, Klinkenberg M, Wu BM I. Mutational analysis of a patient with mucopolysaccharidosis type VII and identification of pseudogenes. *Am J Hum Genet* 1993; **52**: 517.
12. Tomatsu S, Sukegawa K, Ikedo Y *et al.* Molecular basis of mucopolysaccharidosis type VII: replacement of Ala619 in β -glucuronidase with Val. *Gene* 1990; **89**: 283.
13. Tomatsu S, Fukuda S, Sukegawa K *et al.* Mucopolysaccharidosis type VII: characterization of mutations and molecular heterogeneity. *Am J Hum Genet* 1991; **48**: 89.
14. Sly WS. The mucopolysaccharidoses. In: Bondy PK, Rosenberg LE (eds). *Metabolic Control and Disease*. Philadelphia, PA: WB Saunders, 1980: 545.
15. Guibaud P, Maire I, Goddon R *et al.* Mucopolysaccharidose type VII par deficit en β -glucuronidase. Etude d'une famille. *J Genet Hum* 1979; **27**: 29.
16. Hoyne HE, Jones KL, Higginbottom MC, O'Brien JS. Presentation of mucopolysaccharidosis VII (beta-glucuronidase deficiency) in infancy. *J Med Genet* 1981; **18**: 237.
17. Sewell AC, Gehler J, Mittermaier G, Meyer E. Mucopolysaccharidosis type VII (β -glucuronidase deficiency): a report of a new case and a survey of those in the literature. *Clin Genet* 1982; **21**: 366.
18. Gehler J, Cantz M, Tolksdorf M *et al.* Mucopolysaccharidosis VII: β -glucuronidase deficiency. *Humangenetik* 1974; **23**: 149.

19. Beighton P, McKusick VA. *Heritable Disorders of Connective Tissue*, 5th edn. St Louis: CV Mosby, 1993.
20. Wilson D, Melnik E, Sly W, Makesby WR. Neonatal β -glucuronidase deficiency mucopolysaccharidosis (MPS VII): autopsy findings. *J Neuropathol Exp Neurol* 1982; **41**: 344.
21. Irani D, Kim HS, El-Hibri H *et al*. Postmortem observations on beta-glucuronidase deficiency presenting as hydrops fetalis. *Ann Neurol* 1983; **14**: 486.
22. Kagie MJ, Kleijer WJ, Huijman JGM *et al*. Beta-glucuronidase deficiency as a cause of fetal hydrops. *Am J Med Genet* 1992; **42**: 693.
23. Stangenberg M, Lingman G, Roberts G, Ozand P. Mucopolysaccharidosis VII as a cause of fetal hydrops in early pregnancy. *Am J Med Genet* 1992; **15**: 142.
24. Machin GA. Hydrops revisited: literature review of 1414 cases published in 1980s. *Am J Med Genet* 1989; **34**: 366.
25. Molyneux AJ, Blair E, Coleman N, Daish P. Mucopolysaccharidosis type VII associated with hydrops fetalis: histopathological and ultrastructural features with genetic implications. *J Clin Pathol* 1997; **50**: 252.
26. Vervoort R, Islam MR, Sly WS *et al*. Molecular analysis of patients with beta-glucuronidase deficiency presenting as hydrops fetalis or as early mucopolysaccharidosis VII. *Am J Hum Genet* 1996; **58**: 457.
27. Nelson J, Kenny B, O'Hara D *et al*. Foamy changes of placental cells in probable beta glucuronidase deficiency associated with hydrops fetalis. *J Clin Pathol* 1993; **46**: 370.
28. Danes BS, Degnan M. Different clinical and biochemical phenotypes associated with β -glucuronidase deficiency. In: Bergsma D (ed.). *Skeletal Dysplasias*. Birth Defects, Ser X, No 12. New York: National Foundation, March of Dimes, 1974: 251.
29. Pfeiffer RA, Kresse H, Baumer N, Sattinger E. Beta-glucuronidase deficiency in a girl with unusual clinical features. *Eur J Pediatr* 1977; **126**: 155.
30. de Kremer RD, Givogri I, Argarana CE *et al*. Mucopolysaccharidosis type VII (beta-glucuronidase deficiency): a chronic variant with an oligosymptomatic severe skeletal dysplasia. *Am J Med Genet* 1992; **44**: 145.
31. Gitzelmann R, Wiesmann UN, Spycher MA *et al*. Unusually mild course of beta-glucuronidase deficiency in two brothers (mucopolysaccharidosis VII). *Helv Paediatr Acta* 1978; **33**: 413.
32. Lowry RB, Renwick DH. Relative frequency of the Hurler and Hunter syndromes. *N Engl J Med* 1971; **284**: 221.
33. Hall CW, Cantz M, Neufeld EF. A β -glucuronidase deficiency mucopolysaccharidosis: studies in cultured fibroblasts. *Arch Biochem Biophys* 1973; **155**: 32.
34. Erickson AH, Blobel G. Carboxyl-terminal proteolytic processing during biosynthesis of the lysosomal enzymes β -glucuronidase and cathepsin D. *Biochemistry* 1983; **22**: 5201.
35. Bell CE Jr, Sly WS, Brot FE. Human β -glucuronidase deficiency mucopolysaccharidosis; identification of cross-reactive antigen in cultured fibroblasts of deficient patients by enzyme immunoassay. *J Clin Invest* 1977; **59**: 97.
36. Vervoort R, Islam MR, Sly W *et al*. A pseudodeficiency allele (D152 N) of the human beta-glucuronidase gene. *Am J Hum Genet* 1995; **57**: 798.
37. Vervoort R, Gitzelmann R, Bosshard N *et al*. Low beta-glucuronidase enzyme activity and mutations in the human beta-glucuronidase gene in mild mucopolysaccharidosis type VII pseudodeficiency and a heterozygote. *Hum Genet* 1998; **102**: 69.
38. Yamada S, Tomatsu S, Sly WS *et al*. Four novel mutations in mucopolysaccharidosis type VII including a unique base substitution in exon 10 of the beta-glucuronidase gene that creates a novel 5'-splice site. *Hum Mol Genet* 1995; **4**: 651.
39. Lissens W, Dedobbeleer G, Foulon W *et al*. Beta-glucuronidase deficiency as a cause of prenatally diagnosed non-immune hydrops fetalis. *Prenatal Diag* 1991; **11**: 405.
40. Vervoort R, Buist NRM, Kleijer WJ *et al*. Molecular analysis of the beta-glucuronidase gene: novel mutations in mucopolysaccharidosis type VII and heterogeneity of the polyadenylation region. *Hum Genet* 1997; **99**: 462.
41. Sands MS, Barker JE, Vogler C *et al*. Treatment of mucopolysaccharidosis type VII by syngeneic bone marrow transplantation in neonates. *Lab Invest* 1993; **68**: 676.
42. Haskins ME, Desnick RJ, Di Ferrante N *et al*. β -Glucuronidase deficiency in a dog: a model of human mucopolysaccharidosis VII. *Pediatr Res* 1984; **18**: 980.
43. Moullier P, Bohl D, Heard JM, Danos O. Correction of lysosomal storage in the liver and spleen of MPS VII mice by implantation of genetically modified skin fibroblasts. *Nat Genet* 1993; **4**: 154.

MUCOLIPIDOSES

83.	I-cell disease/mucopolipidosis II	613
84.	Mucopolipidosis III/pseudo-Hurler polydystrophy/N-acetyl-glucosaminyl-I-phosphotransferase deficiency	621

I-cell disease/mucopolipidosis II

Introduction	613	Treatment	618
Clinical abnormalities	614	References	618
Genetics and pathogenesis	617		

MAJOR PHENOTYPIC EXPRESSION

Coarse features, shortness of stature, progressive developmental impairment, limitation of joint motion, dysostosis multiplex, cytoplasmic inclusions in fibroblasts, deficient intracellular activity of many hydrolases, and elevated activity of these enzymes in serum because of defective post-translational modification of acid hydrolases, a consequence of the fundamental defect in N-acetylglucosaminyl-(GlcNAc) 1-phosphotransferase.

INTRODUCTION

I-cell disease was first described by Leroy and Demars in 1967 [1]. Cultured fibroblasts derived from the skin of their two patients contained striking cytoplasmic inclusions visible by phase contrast microscopy (Figure 83.1). The patients resembled those with Hurler syndrome, but they presented earlier, did not usually have cloudy corneas, and they did not have increased urinary excretion of mucopolysaccharides. Leroy and colleagues [2] named the disorder I-cell disease, the ‘I’ indicating inclusions. It has since been designated a mucopolipidosis [3], because of the coexistence of abnormalities typical of both mucopolysaccharidoses and sphingolidoses. Leroy [4] has proposed the designation of these disorders as oligosaccharidoses, since large quantities of oligosaccharides are excreted in the urine [5]. The molecular defect has now been defined in N-acetylglucosaminyl-1-phosphotransferase [6] (Figure 83.2) (GlcNAc phosphotransferase). The same enzyme is defective in mucopolipidosis III (Chapter 84). These two disorders represent a unique mechanism of disease in which the basic defect is in the processing of lysosomal enzymes to permit their recognition and uptake into cells [7].

GlcNAc phosphotransferase has a three subunit structure – $\alpha_2\beta_2\gamma_2$ – that is coded for by two genes, one for the α and β subunits and another for the γ [8]. The α/β B gene is located on chromosome 12p [9]. The γ gene is on 16p [10]. Patients with mucopolipidosis II have been found



Figure 83.1 I-cell in fibroblast culture illustrating the characteristic cytoplasmic inclusions. (Courtesy of Dr Jules Leroy, State University of Antwerp, Belgium.)

to have no α/β mRNA, while reduced amounts were found in patients with mucopolipidosis III [9], although the 22 exons of the gene could be amplified. In other families with mucopolipidosis III, a mutation was found in the γ gene, a

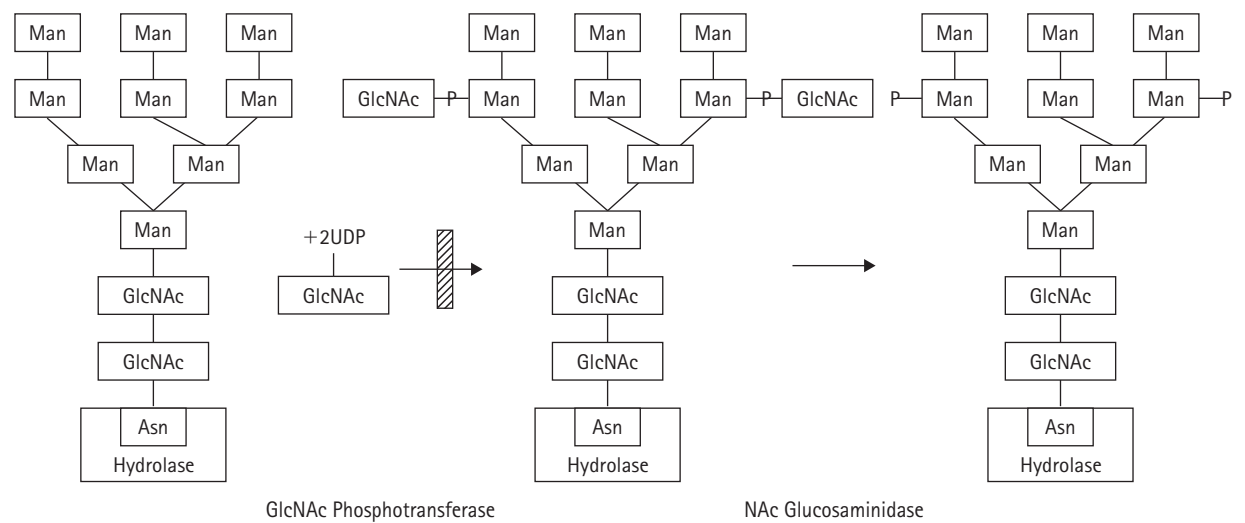


Figure 83.2 N-acetylglucosamine (GlcNAc) phosphotransferase, the site of the defect in I-cell disease and in mucopolidoses III. The pathway for phosphorylating the acid hydrolase enzymes is shown as a two-step reaction, which ultimately forms the mannose-6-phosphate recognition site that targets the enzyme for cellular uptake. Abbreviations employed in addition to GINAc:UDP-GlcNAc for uridine diphosphate-GlcNAc; Man, mannose; and Asn to indicate the linkage of the oligosaccharide to an asparagine residue of the enzyme protein.



Figure 83.3 FSY: A 19-month-old female infant with I-cell disease. She was small and developmentally delayed. She had an umbilical hernia. An inguinal hernia was repaired at four months. The liver was palpable 2 cm below the costal margin. Her sister had the disease.



Figure 83.4 FSY: The features were coarse. The nasal bridge was depressed. She was hirsute and the hairline was low. The head was small.

cytosine insertion at codon 167, which causes a frameshift and a premature termination [10].

CLINICAL ABNORMALITIES

The typical phenotype of the patient with I-cell disease is that of an earlier-onset Hurler syndrome [2, 11–13] (Figures 83.3, 83.4, 83.5, 83.6, 83.7, 83.8, 83.9, 83.10, and 83.11). Findings present at birth include dislocation of the hips, hernias and talipes equinovarus, as well as coarse features [14]. Neonatal cholestatic jaundice has also been reported in an infant with I-cell disease [15].

Impaired psychomotor development is profound and progressive. Patients do not learn to sit, walk, roll over



Figure 83.5 FSY: Coarse features and hirsutism are well visualized in profile.

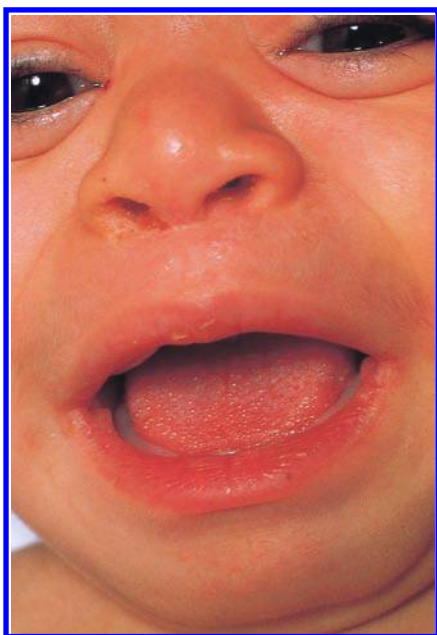


Figure 83.6 FSY: She had gingival hypertrophy. Corneas were not cloudy. The tongue appeared large.



Figure 83.8 ASY: She already had coarse features and gingival hypertrophy. The corneas were clear.



Figure 83.7 ASY: The 5-week-old sister with I-cell disease. In addition, she had congenital heart disease. She had delayed linear growth and the head was small.



Figure 83.9 An infant with I-cell disease illustrating the gingival hyperplasia. (Illustration was kindly provided by Dr Philip Benson.)

or speak. The IQ is very low. Impairment of linear growth is impressive: most patients reach a maximal height of 0.74–0.76 m by two years of age. In contrast in Hurler disease, the final height is 1.0–1.1 m. Coarse features are evident very early, and features are progressively coarser as additional mucopolysaccharide is deposited in bones and soft tissues. The ears are thick and firm. The skin is thick, smooth and firm; it may be so tight that it cannot be

pinched. The forehead is high and narrow with a prominent metopic ridge (Figure 83.10). There are epicanthal folds and puffy eyelids. The bridge of the nose is flat, the tip of the nose wide and the nostrils are anteverted. The filtrum is long. The corneas are characteristically clear, but slit lamp examination may reveal a fine granularity [16], and there may be corneal opacity [17]. There is very prominent gingival hypertrophy [18] (Figure 83.9) – this is a difference from Hurler disease. The voice is hoarse.

Limitation of joint motion is characteristic and contractures may develop at the hips, knees, shoulders, elbows, and fingers. The claw-hand deformity may be



Figure 83.10 A seven-year-old girl with I-cell disease. This was the original patient studied by Dr Jules Leroy [1]. By this age, she had flexion contractures of the fingers, hips, and knees. Her height of 30 inches was equivalent to that of a one-year-old. Facial features were quite like those of a patient with the Hurler disease, but the corneas were clear. (Illustration provided by Dr Leroy of Antwerp, Belgium.)



Figure 83.11 Infant with kyphosis, a manifestation of I-cell disease. (Illustration was kindly provided by Dr Philip Benson.)

identical to that of the Hurler patient. The hands tend to deviate in an ulnar direction. There is a dorsolumbar kyphosis and a lumbar gibbus. The abdomen is protuberant and may contain a pronounced diastasis recti and an umbilical hernia. Inguinal hernias are common in males. Hepatomegaly is minimal, and splenomegaly is slight or absent.

A nasal discharge is usually present. Respiratory infections and otitis media are common. Cardiomegaly may be present early in life [19]. Most patients die between two and eight years of age, usually of pneumonia or

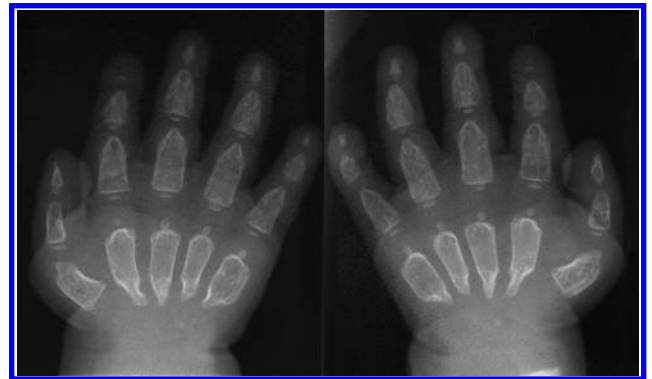


Figure 83.12 Roentgenogram of the hand of a one-month-old patient with I-cell disease illustrates the typical appearance of dysostosis multiplex. The phalanges were short and thick. The metacarpals were broad distally and tapered proximally. The bones had a coarse trabecular pattern.



Figure 83.13 Roentgenograph of the arms of the same patient illustrate the thick bone with poor modeling. The radius and ulna were angulated toward each other.



Figure 83.14 Roentgenograph of the same patient illustrates the broadened spatulate ribs.

congestive cardiac failure [20, 21]. Longer survivors may turn out to be examples of mucopolidosis III.

Roentgenographic features are those of dysostosis multiplex (Figures 83.12, 83.13, and 83.14). They appear at an early age and are similar to those of GM₁ gangliosidosis (Chapter 91) or Hurler disease (Chapter 76). Skeletal changes are present at birth and may be extreme in infancy. Extensive periosteal new bone function produces cloaking and loss of tubulation of the long bones [11]. Premature synostosis of the cranial sutures may be observed as early as one month of age [22]. Changes in the bones are progressive. The long bones are short, wide, and thick. The distal radius and ulna tilt toward each other. Bullet-shaped proximal phalanges are associated with proximal pointing of metacarpals and widening of their distal ends. The ribs are broad and spatulate. The vertebral bodies are short and rounded, and there is anterior inferior breaking at L1 and D12. The proximal tibia and fibula may be deeply notched [16].

At necropsy, there may be thickening of the endocardium and myocardium and of the coronary arteries and aorta. The cytoplasmic inclusions in skin fibroblasts (Figure 83.1) are large, dark granules which fill the cytoplasm [1, 17, 23, 24]. Vacuolated lymphocytes may be seen in the blood or bone marrow [25] and vacuolated hepatocytes or hepatic mesenchymal cells are found [24], as well as storage in renal glomerular and tubular epithelium. Typical histologic changes have been seen in a 15-week-old fetus [26] and a placenta at 14 weeks [27]. The inclusions are sudanophilic and positive for acid phosphatase. The nervous system may appear normal, but lamellar bodies have been observed

in spinal ganglia nerves [28] and anterior horn cells [29]. Cardiac muscle is histologically normal: the valves are thickened, and contain vacuolated fibroblasts [29, 30].

GENETICS AND PATHOGENESIS

Cells of patients with I-cell disease are characterized by deficient activity of a large number of lysosomal enzymes [12, 31–33]. These include β -glucuronidase, β -galactosidase, α -mannosidase, α -fucosidase, N-acetyl- β -D-galactosaminidase and arylsulfatase-A. Wiesmann and colleagues [31] found that the activities of the same lysosomal enzymes were high in the medium surrounding cultured I-cell fibroblasts. High levels of activity of lysosomal enzymes are also found in the serum of patients with I-cell disease [34]. These include hexosaminidase A and B and α -galactosidase. These patients do not have excessive excretion of urinary mucopolysaccharides.

The key to these unusual findings was provided by the observation of Hickman and Neufeld [35] that in I-cell disease there is a defect in the uptake and intracellular location of lysosomal enzymes. I-cells absorb purified normal lysosomal enzyme perfectly well, but lysosomal enzymes derived from I-cell patients are not effectively taken up by normal cells [35]. Lysosomal enzymes such as hexosaminidase A are taken up into cells by absorptive pinocytosis. The uptake of these enzymes by cells is a function of the carbohydrate moieties of the enzyme proteins, because oxidation by sodium metaperiodate interferes with uptake without affecting catalytic activity. These enzymes are normally secreted from cells, after which they must be specifically recognized and taken up. The I-cell mutation interferes with formation of the carbohydrate recognition site on the hydrolases. The recognition marker on lysosomal acid hydrolase enzymes is mannose-6-phosphate [36]. Normal cells incorporate ³²P into newly synthesized lysosomal enzymes; fibroblasts from patients with I-cell disease do not [37, 38]. This led to the hypothesis that the defect in I-cell disease was in the biosynthesis of the phosphomannosyl signal that binds to receptors responsible for targeted uptake of the enzymes. The phosphorylation of the enzymes takes place in a two-step process in which N-acetylglucosaminylphosphate is added to mannose residues of the exposed oligosaccharide of the glycoprotein enzyme (Figure 83.2) [7, 39–41].

The enzyme which catalyzes the first reaction is UDP-N-acetylglucosamine:lysosomal enzyme-N-acetylglucosamine-1-phosphotransferase, or N-acetylglucosaminyl phosphotransferase (GlcNAc phosphotransferase) [6, 42–47]. Fibroblasts from patients with I-cell disease are almost completely deficient in the activity of this enzyme [6, 45]. The other enzyme required for the biosynthesis of the mannose-6-phosphate recognition site is a glucosaminidase that catalyzes the removal of the N-acetylglucosamine residue, exposing the mannose-6-phosphate groups [48]. It is formally called N-acetylglucosamine-1-

phosphodiester- α -N-acetyl-glucosaminidase. Its activity is normal or elevated in fibroblasts of patients with I-cell disease. The biosynthesis of lysosomal enzymes takes place in the endoplasmic reticulum, and the enzyme is transported through the Golgi complex, where the transfer of the N-acetylglucosaminylphosphate to the mannose site occurs prior to transport to the lysosome [49]. As a result of the primary defect in GlcNAc phosphotransferase in I-cell disease this process breaks down, and a number of secondary effects occur, such as the deficiency of a number of lysosomal hydrolases. This leads to the storage of a variety of material such as complex lipids and mucopolysaccharides. I-cell lysosomes also accumulate cystine, much like those of patients with cystinosis [50, 51]. In addition, these cells accumulate sialic acid [52]. Patients with mucopolipidosis III, or pseudo-Hurler polydystrophy, have defective activity of the GlcNAc phosphotransferase enzyme (Chapter 84).

Diagnosis is generally made by assay of lysosomal enzymes in cultured fibroblasts, where there is a distinct deficiency [30, 53] or in the plasma or serum, where there is as much as a ten- to 20-fold increase in enzyme activity [31]. Assay of fibroblasts or plasma for glycosylasparaginase has been reported [54] as useful for the diagnosis of I-cell disease. The diagnosis can also be made by assay of the GlcNAc phosphotransferase in leukocytes or cultured fibroblasts [55, 56]. Substrates are commercially available.

Heterogeneity in the mucopolipidoses was documented first in studies of complementation. Instances were reported of complementation between ML II and ML III fibroblasts which is consistent with the enzyme subunit structure and the fact that there are two genes [57]. The α/β gene contains 20 exons and 80 kb [9]. The precursor molecule that is its product undergoes post-translational cleavage to yield the 928 amino acid α unit and the 328 amino acid β unit. The γ gene codes for a protein of 281 amino acids [10]. There are 23 asparagine-linked glycosylation sites among the subunits. Among patients with mucopolipidosis II mutations studied appear to have been small deletions or point mutations because amplification of the individual α/β exons revealed them to be present, although there was no mRNA [9]. The γ gene was transcribed normally.

I-cell disease is transmitted as an autosomal recessive. Multiple siblings, both male and female, have been reported from families with normal parents. Consanguinity has been documented [3, 16]. Abnormal inclusions have been found in the fibroblasts of some phenotypically normal parents [2]. Obligate heterozygotes have been found to have intermediate levels of the GlcNAc phosphotransferase enzyme in leukocytes and cultured fibroblasts [55, 56]. Prenatal diagnosis of I-cell disease has been carried out by the demonstration of high levels of multiple acid hydrolases in amniotic fluid or their deficiency in cultured amniocytes, as well as by demonstrating accumulation of ^{35}S mucopolysaccharide [26, 58–60]. One affected fetus

has been diagnosed on the basis of hexosaminidase assay of maternal serum [61]. A boy has been described [61] with an atypical form of I-cell disease: he was found to be a mosaic in whom two populations of fibroblasts were demonstrated, one with the characteristic morphology and enzyme defect of I-cell disease, and the other normal.

TREATMENT

Symptomatic treatment (for example, of respiratory infection) is helpful. The natural history is of uniform fatality. Two patients have been treated with bone marrow transplantation [62, 63] with limited improvement.

REFERENCES

1. Leroy JG, Demars RI. Mutant enzymatic and cytological phenotype in cultured human fibroblasts. *Science* 1967; **157**: 804.
2. Leroy JG, Demars RI, Opitz JM. I-cell disease. In: *Proceedings of the First Conference on Clinical Delineation of Birth Defects*. Original Article Series, vol V, No. 4. New York: The National Foundation, 1969: 174.
3. Spranger JW, Wiedemann HR. The genetic mucopolipidoses. *Humangenetik* 1970; **9**: 113.
4. Leroy JG. The oligosaccharidoses: proposal of a new name and a new classification for the mucopolipidoses. In: Nyhan WL, Jones KL (eds). *Dysmorphology*. Birth Defects Original Article Series, vol 18, No 3B. New York: The National Foundation, 1982: 3.
5. Strecker G, Peers MC, Michalski JC *et al*. Structure of nine sialyloligosaccharides accumulated in urine of eleven patients with three different types of sialidosis. *Eur J Biochem* 1977; **75**: 391.
6. Reitman ML, Varki AM, Kornfeld S. Fibroblasts from patients with I-cell disease and pseudo-Hurler polydystrophy are deficient in uridine 5' diphosphate-N-acetylglucosamine: glycoprotein N-acetylglucosaminylphosphotransferase activity. *J Clin Invest* 1981; **67**: 1574.
7. Creek KE, Sly WS. The role of the phosphomannosyl receptor in the transport of acid hydrolases to lysosomes. In: Dingle JR, Dean RT, Sly W (eds). *Lysosomes in Biology and Pathology*. New York: Elsevier/North Holland, 1984: 63.
8. Bao M, Booth JL, Elmendorf BJ, Canfield WM. Bovine UDP-N-acetylglucosamine: lysosomal-enzyme N-acetylglucosamine-1-phosphotransferase: I Purification and subunit structure. *J Biol Chem* 1996; **271**: 31437.
9. Canfield W, Bao M, Pan J *et al*. Mucopolipidosis II and mucopolipidosis IIIA are caused by mutations in the GlcNAc-phosphotransferase α/β gene on chromosome 12. *Am J Hum Genet* 1998; **63**: A15.
10. Raas-Rothschild A, Cormier-Daire V, Bao M *et al*. Truncation of the UDP-N-acetylglucosamine: Lysosomal enzyme N-acetylglucosamine-1-phosphotransferase γ -subunit gene causes variant mucopolipidosis III (pseudo-Hurler polydystrophy). *J Clin Invest* 2000; **105**: 673.

11. Leroy JG, Spranger JW, Feingold M *et al.* I-cell disease: a clinical picture. *J Pediatr* 1971; **79**: 360.
12. Leroy JG, Spranger JW. I-cell disease. *N Engl J Med* 1970; **283**: 598.
13. Luchsinger U, Buhler EM, Mehes K, Hirt HR. I-cell disease. *N Engl J Med* 1970; **282**: 1374 (letter).
14. Cipolloni C, Boldrini A, Dontie E *et al.* Neonatal mucopolipidosis II (I-cell disease): clinical radiological and biochemical studies in a case. *Helv Paediatr Acta* 1980; **35**: 85.
15. Hochman JA, Treem WR, Dougherty F, Bentley RC. Mucopolipidosis II (I-cell disease) presenting as neonatal cholestasis. *J Inherit Metab Dis* 2001; **24**: 603.
16. Blank E, Linder D. I-cell disease (mucopolipidosis II): a lysosomopathy. *Pediatrics* 1974; **54**: 797.
17. deMontis G, Garnier P, Thomassin N *et al.* La mucopolipidose type II (maladie des cellules a inclusions). Etude d'un cas et revue de la literature. *Ann Pediatr* 1972; **19**: 369.
18. Taylor NG, Shuff RY. I-cell disease: an unusual cause of gingival enlargement. *Br Dent J* 1994; **176**: 106.
19. Spritz RA, Doughty RA, Spackman TJ *et al.* Neonatal presentation of I-cell disease. *J Pediatr* 1978; **93**: 954.
20. Okada S, Owada M, Sakiyama T *et al.* I-cell disease: clinical studies of 21 Japanese cases. *Clin Genet* 1985; **28**: 207.
21. Satoh Y, Sakamoto K, Fujibayashi Y *et al.* Cardiac involvement in mucopolipidosis: importance of non-invasive studies for detection of cardiac abnormalities. *Jpn Heart J* 1983; **24**: 149.
22. Patriquin HB, Kaplan P, Kind HP, Giedion A. Neonatal mucopolipidosis I (I-cell disease) clinical and radiologic features in three cases. *Am J Roentgenol* 1977; **129**: 37.
23. Hanai J, Leroy JG, O'Brien JS. Ultrastructure of cultured fibroblasts in I-cell disease. *Am J Dis Child* 1971; **122**: 34.
24. Kenyon KR, Sensebrenner JA, Wylie RG. Hepatic ultrastructure and histochemistry in mucopolipidosis II (I-cell disease). *Pediatr Res* 1973; **7**: 560.
25. Rapola J, Autio S, Aula P, Nanto V. Lymphocytic inclusions in I-cell disease. *J Pediatr* 1974; **85**: 88.
26. Aula P, Rapola J, Autio S *et al.* Prenatal diagnosis and fetal pathology of I-cell disease (mucopolipidosis type II). *J Pediatr* 1975; **87**: 221.
27. Rapola J, Aula P. Morphology of the placenta in fetal I-cell disease. *Clin Genet* 1977; **11**: 107.
28. Nagashima K, Sakakibara K, Endo H *et al.* I-cell disease (mucopolipidosis II): pathological and biochemical studies of an autopsy case. *Acta Pathol Jpn* 1977; **27**: 251.
29. Martin JJ, Leroy JG, Van Eygen M, Ceuterick C. I-cell disease: a further report on its pathology. *Acta Neuropathol (Berl)* 1984; **64**: 234.
30. Martin JJ, Leroy JG, Farriaux JP *et al.* I-cell disease (mucopolipidosis II). *Acta Neuropathol (Berl)* 1975; **33**: 285.
31. Wiesmann UN, Lightbody J, Vasella F, Herschkowitz NN. Multiple lysosomal enzyme deficiency due to enzyme leakage? *N Engl J Med* 1971; **284**: 109.
32. Wiesmann UN, Herschkowitz NN. Studies on the pathogenic mechanism of I-cell disease in cultured fibroblasts. *Pediatr Res* 1974; **8**: 965.
33. Leroy JG, Ho MW, MacBrinn MC *et al.* I-cell disease; biochemical studies. *Pediatr Res* 1972; **6**: 752.
34. Wiesmann UN, Vasella F, Herschkowitz NN. I cell disease. Leakage of lysosomal enzymes into extracellular fluids. *N Engl J Med* 1971; **28**: 1090.
35. Hickman S, Neufeld EF. A hypothesis for I-cell disease: defective hydrolases that do not enter lysosomes. *Biochem Biophys Res Commun* 1972; **49**: 992.
36. Natowicz MR, Chi MY-Y, Lowry OH, Sly WS. Enzymatic identification of mannose 6-phosphate on the recognition marker for receptor-mediated pinocytosis of β -glucuronidase by human fibroblasts. *Proc Natl Acad Sci USA* 1979; **76**: 4322.
37. Hasilik A, Neufeld EF. Biosynthesis of lysosomal enzymes in fibroblasts; phosphorylation of mannose residues. *J Biol Chem* 1980; **255**: 4946.
38. Bach G, Barga R, Cantz M. I-cell disease: deficiency of extracellular hydrolase phosphorylation. *Biochem Biophys Res Commun* 1979; **91**: 476.
39. Tabas I, Kornfeld S. Biosynthetic intermediates of β -glucuronidase contain high mannose oligosaccharides with blocked phosphate residues. *J Biol Chem* 1980; **255**: 6633.
40. Varki A, Kornfeld S. Structural studies of phosphorylated high mannose-type oligosaccharides. *J Biol Chem* 1980; **255**: 10847.
41. Hasilik A, Klein U, Waheed A *et al.* Phosphorylated oligosaccharides in lysosomal enzymes: identification of α -N-acetylglucosamine(1)phospho(6)mannose diester groups. *Proc Natl Acad Sci USA* 1980; **77**: 7074.
42. Reitman ML, Kornfeld S. Lysosomal enzyme targeting: N-acetylglucosaminylphosphotransferase selectively phosphorylates native lysosomal enzymes. *J Biol Chem* 1981; **256**: 11977.
43. Waheed A, Hasilik A, Von Figura K. UDP-N-acetylglucosamine: lysosomal enzyme precursor N-acetylglucosamine-1-phosphotransferase: partial purification and characterization of the rat liver Golgi enzyme. *J Biol Chem* 1982; **257**: 12322.
44. Lang L, Reitman M, Tang J *et al.* Lysosomal enzyme phosphorylation: recognition of a protein-dependent determinant allows specific phosphorylation of oligosaccharides present on lysosomal enzymes. *J Biol Chem* 1984; **259**: 14663.
45. Hasilik A, Waheed A, Von Figura K. Enzymatic phosphorylation of lysosomal enzymes in the presence of UDP-N-acetylglucosamine. Absence of the activity in I-cell fibroblasts. *Biochem Biophys Res Commun* 1981; **98**: 761.
46. Waheed A, Pohlmann R, Hasilik A, Von Figura K. Subcellular location of two enzymes involved in the synthesis of phosphorylated recognition markers in lysosomal enzymes. *J Biol Chem* 1981; **256**: 4150.
47. Reitman ML, Kornfeld S. UDP-N-acetylglucosamine: glycoprotein N-acetyl-glucosamine-1-phosphotransferase. Proposed enzyme for the phosphorylation of the high mannose oligosaccharide units of lysosomal enzymes. *J Biol Chem* 1981; **256**: 4275.
48. Varki A, Sherman N, Kornfeld S. Demonstration of the enzymatic mechanisms of α -N-acetyl-D-glucosamine-1-phosphodiester N-acetylglucosaminidase (formerly called α -N-acetylglucosaminylphosphodiesterase) and

- lysosomal-N-acetylglucosaminidase. *Arch Biochem Biophys* 1983; **222**: 145.
49. Fischer HD, Gonzalez-Noriega A, Sly WS, Morre DJ. Phosphomannosyl-enzyme receptors in rat liver. Subcellular distribution and role in intracellular transport of lysosomal enzymes. *J Biol Chem* 1980; **255**: 9608.
50. Greene AA, Jonas AJ, Harms E *et al*. Lysosomal cystine storage in cystinosis and mucopolipidosis type II. *Pediatr Res* 1985; **19**: 1170.
51. Tietze F, Rome LH, Butler JD. Impaired clearance of free cystine from lysosome-enriched granular fractions of I-cell disease fibroblasts. *Biochem J* 1986; **237**: 9.
52. Vladutiu GD, Fike RM, Amigone VT. Influence of sialic acid on cell surface properties in I-cell disease fibroblasts. *In Vitro* 1981; **17**: 588.
53. Hall CW, Liebaers I, DiNatale P, Neufeld EF. Enzymatic diagnosis of the genetic mucopolysaccharide storage disorders. *Methods Enzymol* 1978; **50**: 439.
54. Ylikangas PK, Mononen IT. Glycosylasparaginase as a marker enzyme in the detection of I-cell disease. *Clin Chem* 1988; **44**: 2543.
55. Varki A, Reitman ML, Vannirt S *et al*. Demonstration of the heterozygous state for I-cell disease and pseudo-Hurler polydystrophy by assay of N-acetylglucosaminylphosphotransferase in white blood cells and fibroblasts. *Am J Hum Genet* 1982; **34**: 717.
56. Mueller OT, Little LE, Miller AL *et al*. I-cell disease and pseudo-Hurler polydystrophy: heterozygote detection and characteristics of the altered N-acetylglucosaminylphosphotransferase in genetic variants. *Clin Chim Acta* 1985; **150**: 175.
57. Mueller OT, Honey NK, Little LE *et al*. Mucopolipidosis II and III: the genetic relationship between two disorders of lysosomal enzyme biosynthesis. *J Clin Invest* 1983; **72**: 1016.
58. Hujing F, Warren RJ, McLeod AGW. Elevated activity of lysosomal enzymes in amniotic fluid of a fetus with mucopolipidosis II (I-cell disease). *Clin Chim Acta* 1973; **44**: 453.
59. Matsuda I, Arashum S, Mitsuyama T *et al*. Prenatal diagnosis of I-cell disease. *Hum Genet* 1975; **30**: 69.
60. Gehler J, Cantz M, Stoeckenius M, Spranger J. Prenatal diagnosis of mucopolipidosis II (I-cell disease). *Eur J Pediatr* 1976; **122**: 201.
61. Hug G, Bove KE, Soukup S *et al*. Increase serum hexosaminidase in a woman pregnant with a fetus affected by mucopolipidosis II (I-cell disease). *N Engl J Med* 1984; **311**: 988.
62. Kurobane I, Inoue S, Gotoh YH *et al*. Biochemical improvement after treatment by bone marrow transplantation in I-cell disease. *Tohoku J Exp Med* 1986; **150**: 63.
63. Imaizumi M, Gushi K, Kurobane I *et al*. Long-term effects of bone marrow transplantation for inborn errors of metabolism: a study of four patients with lysosomal storage diseases. *Acta Paediatr Jpn* 1994; **36**: 30.

Mucopolipidosis III/pseudo-Hurler polydystrophy/ N-acetyl-glucosaminyl-1-phosphotransferase deficiency

Introduction	621	Treatment	626
Clinical abnormalities	621	References	626
Genetics and pathogenesis	625		

MAJOR PHENOTYPIC EXPRESSION

Joint pain, stiffness, contractures; shortness of stature; malocclusion, gingival hypertrophy; aortic diastolic murmur; dysostosis multiplex, and deficiency of GlcNAc phosphotransferase.

INTRODUCTION

Mucopolipidosis II and III reflect multiple deficiencies of many lysosomal hydrolases that require post-translational processing to form the recognition site that permits their cellular uptake. The fundamental defect is in N-acetylglucosaminyl-1-phosphotransferase (GlcNAc phosphotransferase) (see [Chapter 83](#), Figure 83.2) [1]. The lysosomal enzyme substrates for this enzyme are glycoproteins containing reactive mannose molecules and in the reaction a GlcNAc phosphate is linked to the mannose; a subsequent phosphodiesterase reaction cleaves off the GlcNAc, leaving the mannose phosphate recognition site. Patients with I-cell disease, or mucopolipidosis II, have complete deficiency of this enzyme, while patients with mucopolipidosis III have varying amounts of residual activity of the enzyme. Variable patterns of clinical phenotype in mucopolipidosis III reflect the considerable variation in enzyme activity as well as its effect on so very many lysosomal enzymes. The extent of the phenotypic variability has doubtless not yet been defined.

There is genetic heterogeneity in mucopolipidosis III caused by the presence of two genes which code for the three subunits of GlcNAc phosphotransferase, α/β and γ [2, 3]. Abnormalities in both genes have been found in different patients with mucopolipidosis III.

CLINICAL ABNORMALITIES

Mucopolipidosis III shares many of the clinical manifestations of the classic mucopolysaccharidoses. In fact, the roentgenographic characteristics are those of a florid dysostosis multiplex ([Figures 84.1, 84.2, 84.3, 83.4](#), and

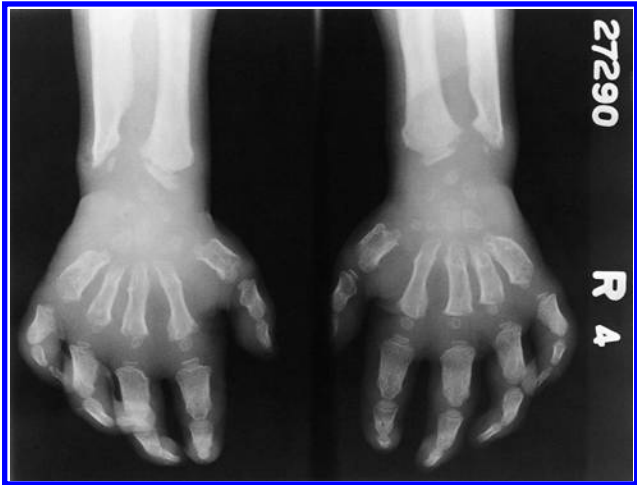


Figure 84.1 Roentgenogram of the hand of an 11-year-old patient with mucopolipidosis type III. The picture was that of an extreme degree of dysostosis multiplex.

84.5). The films of one of our patients [4] were kept in the teaching file of a medical school department of radiology as exemplifying Hurler disease. The disease was originally described [5] as pseudo-Hurler polydystrophy. There is, however, no mucopolysacchariduria. The long bones are short and thick. The distal radius and ulna tilt toward each other. The proximal phalanges are bullet-shaped and the metacarpals are broad distally and pointed proximally. The ribs are broad and spatulate. Vertebral bodies are short and L1 and T12 may be anteriorly beaked (Figures 84.4 and

84.5). There may be early craniosynostosis (Figure 84.6). In other patients, the skull may be normal. There may be hypoplasia of the odontoid. Degenerative changes of the joints, especially the proximal femoral areas, may be characteristic.



Figure 84.2 Roentgenogram of hand of a ten-month-old infant with mucopolipidosis III. There was extreme osteopenia with a fine inner reticular pattern. The phalanges were bullet-shaped. The metacarpals were broad at their distal ends and tapered proximally. The radius and ulna were angulated toward each other.

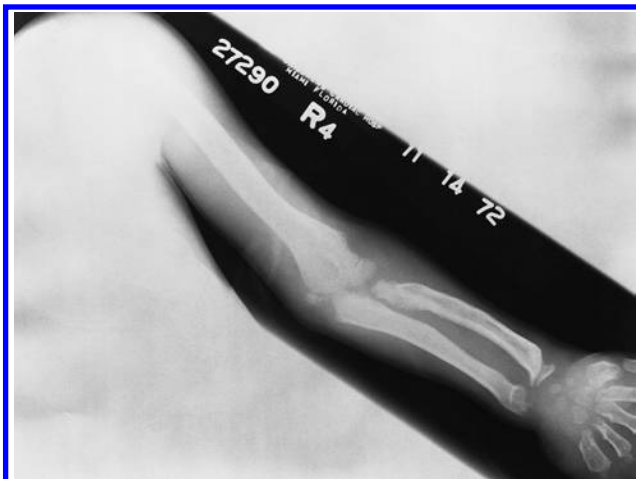


Figure 84.3 Roentgenogram of the radius and ulna of a patient with mucopolipidosis III.



Figure 84.4 Roentgenographic appearance of the broad spatulate ribs and of the spine of a patient with mucopolipidosis III.



Figure 84.5 Roentgenographic appearance of the broad spatulate ribs and of the spine of another patient with mucopolipidosis III.

Patients usually present between two and four years of age with symptoms referable to the joints [4]. Pain is severe enough to awaken them from sleep. Tenderness and early progressive stiffness and limitation of motion may lead to a presumptive diagnosis of juvenile rheumatoid arthritis, but the sedimentation rate remains normal [6]. All of the joints may be involved, and most patients develop some contractures. The claw hand that results by six years of age may be indistinguishable from that of the patient with Hurler disease (Figure 84.7). In some patients, the hands may be quite different, with prominent joints but elongated digits without contracture (Figure 84.8).

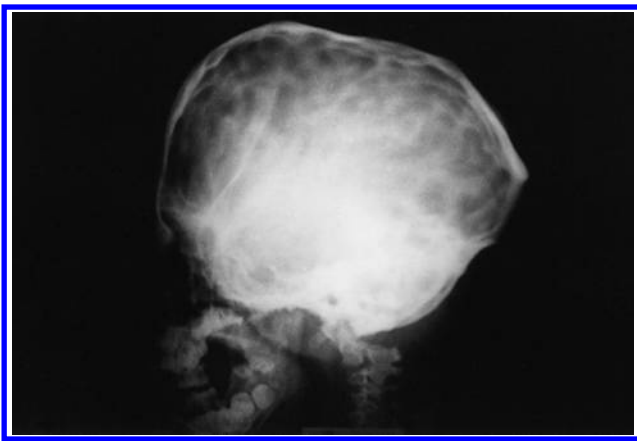


Figure 84.6 Roentgenogram of the skull of the patient in Figure 84.5 illustrates the shape and beaten silver appearance of premature craniosynostosis.

A carpal tunnel syndrome may develop. Contractures of the knees, hips, and elbows are in flexion, leading to a jockey-like appearance (Figure 84.9). These changes lead regularly to shortness of stature (Figure 84.10), but this may be variable. Females may be taller and less severely affected than males [7, 8]. Progressive destructive changes in the hip may lead to a waddling gait and compromised mobility. Rarely, isolated involvement of the hip and spine may be the only clinical manifestations [9]. Late effects are destruction of the femoral heads and of vertebral bodies.

The facial features may also be sufficiently coarse to suggest a diagnosis of mucopolysaccharidosis (Figures



Figure 84.8 The hand of the infant shows periarticular swelling and limitation of joint motion.



Figure 84.7 The claw hand deformity may be identical to that of Hurler syndrome.

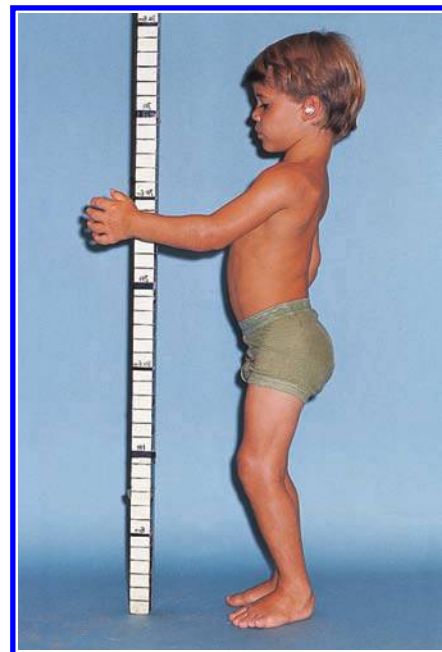


Figure 84.9 An 11-year-old boy with mucopolipidosis III. Contractures of the knees and hips and exaggerated lordosis give a bent position.

84.10, 84.11, 84.12, and 84.13), but in some patients the face may appear normal (Figure 84.9). Hirsutism may be prominent and there may be synophris. The appearance of the mouth may be characteristic, with gingival hypertrophy and crowding of teeth with malocclusion (Figures 84.14 and 84.15). Gingival hypertrophy is always seen in I-cell

disease (mucopolipidosis II). The skin may become thickened. The corneas may appear normal, but steaminess of the cornea may require a slit lamp for visualization, or it may be evident to the naked eye, as in Hurler or Maroteaux-Lamy syndromes. In some, slit lamp examination may be normal. Hyperopic astigmatism and mild retinopathy

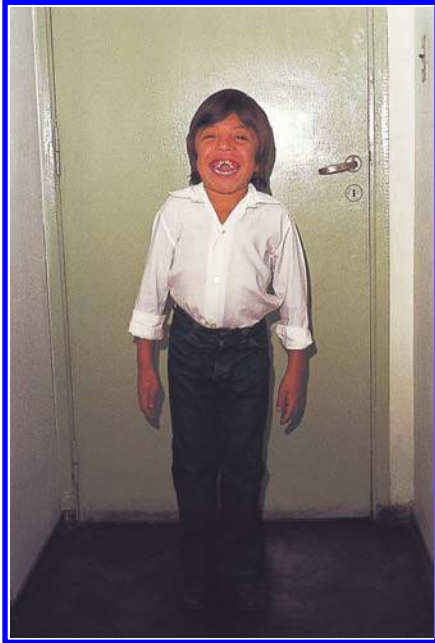


Figure 84.10 A ten-year-old boy with mucopolipidosis was short and had limitation of motion at the elbows, knees, and hands.



Figure 84.12 RV: A one-month-old infant with mucopolipidosis III. She was developmentally delayed and had coarse features. The position of the legs was in treatment of bilateral dislocations of the hips.

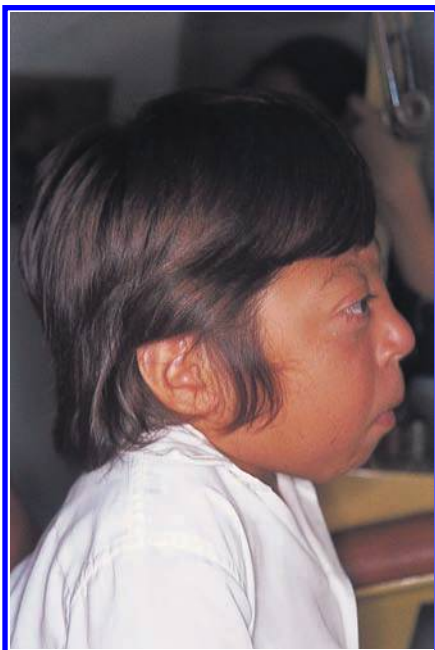


Figure 84.11 Facial features of this patient were coarse and the eyes prominent.



Figure 84.13 Close up of the face illustrates the coarse features indicative of storage of mucopolysaccharide within the skin, illustrated for example in the nose.



Figure 84.14 The hyperplasia of the gums was well delineated by ten months; it had been noted by the parents at birth. The gingiva was also cleft.



Figure 84.15 Gingival hypertrophy and malocclusion were prominent features in this patient.

have also been described [10]. Intelligence may be normal [4], but most patients with mucopolipidosis III have some limitation in cognitive function. IQ levels of 70–90 are commonly encountered. In contrast, patients with I-cell disease have degrees of impairment incompatible with walking or talking. Infiltration of the endocardium may lead to aortic regurgitation and its characteristic diastolic murmur by the end of the first decade, but symptoms of cardiac insufficiency are rare. The liver is only moderately enlarged [11]. Some patients have inguinal hernias. Intelligence is usually to some extent compromised, but not severely [4, 8]. Life expectancy is appreciably better than in mucopolipidosis II, and survival to adulthood is not uncommon [8, 12].

The histologic characteristic of these mucopolipidoses is the appearance of cytoplasmic inclusion bodies [8]. These dense bodies, seen in cultured fibroblasts on phase

microscopy (Chapter 83), are the inclusions that gave I-cell disease its first name. Inclusions or vacuolation may be seen in other cells, such as biopsied cornea, bone marrow cells, or lymphocytes [13, 14].

Clinical features of patients with defects limited to the γ -subunit tend to be somewhat milder. Recognition of symptoms may be in the latter half of the first decade of life [15, 16], and generally there is normal psychomotor development. The problems are mostly orthopedic, including dysostosis, joint stiffness, and joint pain.

GENETICS AND PATHOGENESIS

Specific biochemical diagnosis is often first suggested when fibroblasts or lymphocytes are assayed for the activity of lysosomal hydrolase enzymes. Defective activity is demonstrable for a number of different enzymes, such as hexosaminidase, glucuronidase, and arylsulfatase A [8]. Activities of the same enzymes are high in the media in which the cells are grown [17], which suggested at first that the cells were leaky. These same enzymes may be found in high levels of activity in the serum of patients. Activities may be 100-fold the normal level for some enzymes. The reason for this is not leaky cells but abnormal lysosomal enzymes, which are normally secreted and then avidly taken up. Mucopolipidosis III enzymes cannot be taken up by normal cells while enzymes from normal cells are taken up normally by mucopolipidosis cells [18]. This is because normal enzymes have the mannose-6-phosphate recognition marker that is essential for normal transport of the enzyme, and those of mucopolipidosis patients are deficient in this phosphomannosyl signal (see Chapter 83, Figure 83.2). Activities of β -glucosidase in fibroblasts of patients are normal, because this enzyme is targeted to lysosomes by a phosphorylation-independent mechanism [19].

The enzyme that catalyzes the initial phosphorylation of mannose residues in the glycoprotein enzymes is formally UDP-N-acetylglucosamine:lysosomal enzyme-N-acetylglucosamine-1-phosphotransferase; because its other substrate is UDP-N-acetylglucosamine, we have shortened this to GlcNAc phosphotransferase. Somatic cell hybridization studies have revealed distinct complementation groups [20, 21], which are now referred to as groups A, B, and C. Group A is the most common; many I-cell patients also fit into this group [22]. Group C is uncommon and group B is rare. In the phosphotransferase assay, which utilizes α -methylmannoside as acceptor, patients with mucopolipidosis III in the complementation group C have normal enzyme activity [23, 24]. Those of groups A and B display defective activity against all substrates. The enzyme normally has two distinct functions: recognition of and affinity for the lysosomal enzyme protein, and catalytic phosphorylation of mannose residues. In parallel, studies with lysosomal enzymes as substrates and methylmannoside substrate have elucidated the existence of two

distinct groups of patients: one in which activity against both is deficient, and the other in which activity against α -methylmannoside is normal but phosphorylation of lysosomal enzymes is impaired. This would be consistent with specific interference with recognition versus defect in catalytic function [25]. In 2008, Cathey *et al.* [26] proposed an updated nomenclature system, replacing the term ML II with ML II α/β , ML IIIA with ML III α/β , and ML IIIC with ML III γ .

The α/β gene has been mapped to chromosome 12p23.2 [2] and is identified as *GNPTAB*. The γ gene has been mapped to 16p13.3 [3] and is labeled *GNPTG*. Some patients with mucopolipidosis III have been found to have abnormalities in the α/β gene, because there is reduced transcription to mRNA [2]. In other families with mucopolipidosis III, a mutation was found in the γ gene [3]. This insertion of a cytosine at codon 167 leads to a frame shift and a premature termination 107 bp downstream [3]. There are more than 126 known mutations in *GNPTAB*, of which some 38 are publicly listed, and more than 29 mutations in *GNPTG*, of which eight are publicly listed [27].

Genetic transmission is autosomal recessive, as it is in I-cell disease. Consanguinity has been observed [7]. Heterozygotes have been reported to have intermediate levels of GlcNAc phosphotransferase in leukocytes and cultured fibroblasts [23]. Prenatal diagnosis should be possible by assay of the enzyme in cultured amniocytes or chorionic villus cells.

TREATMENT

Supportive orthopedic management and physiotherapy may be useful, especially for abnormalities in the hips. It is recommended that hip surgery is delayed until after puberty. Surgical correction of carpal tunnel syndrome is useful. Intravenous pamidronate has been reported [28] to reduce bone pain and improve mobility, but biochemical, histological, and roentgenographic evidence of bone resorption did not improve.

REFERENCES

1. Reitman ML, Varki A, Kornfeld S. Fibroblasts from patients with I-cell disease and pseudo-Hurler polydystrophy are deficient in uridine 5'-diphosphate-N-acetylglucosamine: glycoprotein acetylglucosaminylphosphotransferase activity. *J Clin Invest* 1981; **67**: 1574.
2. Canfield W, Bao M, Pan J *et al.* Mucopolipidosis II and mucopolipidosis IIIA are caused by mutations in the GlcNAc-phosphotransferase α/β gene on chromosome 12. *Am J Hum Genet* 1998; **63**: A15.
3. Raas-Rothschild A, Cormier-Daire V, Bao M *et al.* Truncation of the UDP-N-acetylglucosamine: lysosomal enzyme N-acetylglucosamine-1-phosphotransferase γ -subunit gene causes variant mucopolipidosis III (pseudo-Hurler polydystrophy). *J Clin Invest* 2000; **105**: 673.
4. O'Brien JS, Nyhan WL, Shear C *et al.* Clinical and biochemical expression of a unique mucopolysaccharidosis. *Clin Genet* 1976; **9**: 399.
5. Maroteaux P, Lamy M. Les dysplasies spondylo-épiphyssaires génétiques. *Semin Hop Paris* 1958; **34**: 1685.
6. Brik R, Mandel H, Aiziil A *et al.* Mucopolipidosis III presenting as a rheumatological disorder. *J Rheumatol* 1993; **20**: 133.
7. Ward C, Singh R, Slade C *et al.* A mild form of mucopolipidosis type III in four Baluch siblings. *Clin Genet* 1993; **44**: 313.
8. Kelly TE, Thomas GH, Taylor HA Jr *et al.* Mucopolipidosis III (pseudo-Hurler polydystrophy): clinical and laboratory studies in a series of 12 patients. *Johns Hopkins Med J* 1975; **137**: 156.
9. Freisinger P, Padovani JC, Maroteaux P. An atypical form of mucopolipidosis III. *J Med Genet* 1992; **29**: 834.
10. Traboulsi M, Maumenee IH. Ophthalmologic findings in mucopolipidosis III (pseudo-Hurler polydystrophy). *Am J Ophthalmol* 1986; **102**: 592.
11. McKusick VA. Mucopolipidosis III (the mucopolysaccharidoses). In: *Heritable Disorders of Connective Tissue*, 4th edn. St Louis: CV Mosby, 1972: 652.
12. Umehara F, Matsumoto W, Kuriyama M *et al.* Mucopolipidosis III (pseudo-Hurler polydystrophy): clinical studies in aged patients in one family. *J Neurol Sci* 1997; **146**: 167.
13. Taylor HA, Thomas GH, Miller CS *et al.* Mucopolipidosis III (pseudo-Hurler polydystrophy): cytological and ultrastructural observations of cultured fibroblast cells. *Clin Genet* 1973; **4**: 388.
14. Stein H, Berman ER, Lioni N *et al.* Pseudo-Hurler polydystrophy (mucopolipidosis III): a clinical biochemical and ultrastructural study. *Isr J Med Sci* 1974; **10**: 463.
15. Encarnação M, Lacerda L, Costa R *et al.* Molecular analysis of the *GNPTAB* and *GNPTG* genes in 13 patients with mucopolipidosis type II or type III – identification of eight novel mutations. *Clin Genet* 2009; **76**: 76–84.
16. Pohl S, Encarnação M, Castrichini M *et al.* Loss of N-acetylglucosamine-1-phosphotransferase gamma subunit due to intronic mutation in *GNPTG* causes mucopolipidosis type III gamma: implications for molecular and cellular diagnostics. *Am J Med Genet A* 2010; **152A**: 124–32.
17. Wiesemann UN, Lightbody J, Vassella F, Hersch-Kowitz NN. Multiple lysosomal enzyme deficiency due to enzyme leakage? *N Engl J Med* 1971; **284**: 109.
18. Hickman S, Neufeld EF. A hypothesis for I-cell disease: defective hydrolases that do not enter lysosomes. *Biochem Biophys Res Commun* 1972; **49**: 992.
19. Aerts JMFG, Schram AW, Strijland A *et al.* Glucocerebrosidase, a lysosomal enzyme that does not undergo oligosaccharide phosphorylation. *Biochem Biophys Acta* 1988; **964**: 303.
20. Honey NK, Mueller OT, Little LB *et al.* Mucopolipidosis III is genetically heterogeneous. *Proc Natl Acad Sci USA* 1982; **79**: 7420.
21. Gravel RA, Gravel YA, Miller AL, Lowden JA. Genetic complementation analysis of I-cell disease and pseudo-Hurler polydystrophy. In: Callahan JW, Lowden JA (eds). *Lysosomes and Lysosomal Storage Diseases*. New York: Raven Press, 1981: 289.

22. Mueller OT, Honey NK, Little LB *et al.* Mucopolipidosis II and III. The genetic relationships between two disorders of lysosomal enzyme biosynthesis. *J Clin Invest* 1983; **72**: 1016.
23. Varki A, Reitman ML, Vannirt S *et al.* Demonstration of the heterozygous state for I-cell disease and pseudo-Hurler polydystrophy by assay of N-acetyl-glucosaminylphosphotransferase in white blood cells and fibroblasts. *Am J Hum Genet* 1982; **34**: 717.
24. Mueller OT, Little LB, Miller AL *et al.* I-cell disease and pseudo-Hurler polydystrophy: heterozygote detection and characteristics of the altered N-acetyl-glucosamine-phosphotransferase in genetic variants. *Clin Chim Acta* 1985; **150**: 176.
25. Varki AP, Reitman ML, Kornfeld S. Identification of a variant of mucopolipidosis III (pseudo-Hurler polydystrophy): a catalytically active N-acetyl-glucosaminylphosphotransferase that fails to phosphorylate lysosomal enzymes. *Proc Natl Acad Sci USA* 1981; **78**: 7773.
26. Cathey SS, Kudo M, Tiede S *et al.* Molecular order in mucopolipidosis II and III nomenclature. *Am J Med Genet A* 2008; **146A**: 512–13.
27. Stenson PD, Ball EV, Howells K *et al.* The Human Gene Mutation Database: providing a comprehensive central mutation database for molecular diagnostics and personalized genomics. *Hum Genom* 2009; **4**: 69–72.
28. Robinson C, Baker N, Noble J *et al.* The osteodystrophy of mucopolipidosis type III and the effects of intravenous pamidronate treatment. *J Inherit Metab Dis* 2002; **25**: 681.

PART 12

DISORDERS OF CHOLESTEROL AND NEUTRAL LIPID METABOLISM

85.	Familial hypercholesterolemia	631
86.	Mevalonic aciduria	642
87.	Lipoprotein lipase deficiency/type I hyperlipoproteinemia	648

Familial hypercholesterolemia

Introduction	631	Treatment	636
Clinical abnormalities	632	References	638
Genetics and pathogenesis	634		

MAJOR PHENOTYPIC EXPRESSION

Xanthomas, coronary artery disease, hypercholesterolemia, elevated concentration of low density lipoprotein (LDL) cholesterol in plasma, and defective LDL receptor activity.

INTRODUCTION

Familial hypercholesterolemia (FH) is an important model disease. The fundamental defect is an abnormality in a receptor molecule [1]. The study of this disease, especially in the homozygous form, has provided insights into the regulation of the metabolism of cholesterol. This has led to practical approaches to the management of the more common heterozygous disease and other forms of hypercholesterolemia. Familial hypercholesterolemia makes for compelling evidence of the causal relationship between elevated levels of cholesterol in the blood and coronary atherosclerosis.

The disease is dominantly expressed in heterozygotes, who develop coronary artery disease after the age of 30 years. In homozygotes, the concentrations of cholesterol in the blood are enormous; coronary artery disease develops in childhood. The genetics were worked out by Khachadurian [2] in Lebanon, where an unusual number of homozygotes and a very high incidence of consanguinity have been observed. Variation at a single gene locus leads to three distinct phenotypes: homozygous affected, heterozygous, and homozygous normal. Familial hypercholesterolemia is heterogeneous genetically. It is caused by mutations in at least three different genes. The most common variant, accounting for approximately 93 percent of patients is caused by mutations in the low density lipoprotein receptor (LDLR); the resultant disease is currently known as familial hypercholesterolemia (FH). Mutations in apolipoprotein B-100 (APOB) account for approximately 5.5 percent of

patients, and this disease is referred to as familial defective APOB (FDB). In approximately 2 percent of patients, the mutation is in the proprotein convertase subtilisin/kexin type 9 gene (*PCSK9*) [3]. Some 1741 mutations have been identified in the gene *LDLR*. Of these, 108 variants were found in Chinese patients [4].

The relationship between familial hypercholesterolemia and LDL was established by the studies of Gofman *et al.* [5] and of Frederickson *et al.* [6] and their colleagues. The nature of the fundamental defect in the receptor, its variety, and the nature of mutation have been laid out in the elegant work of Brown and Goldstein [1, 7] ([Figure 85.1](#)). LDL cholesterol is taken up by cells after binding of the LDL to its receptor in coated pits on the cell surface, which then undergo endocytic internalization. When the receptor is defective, LDL cholesterol cannot be removed from the plasma, levels are very high, and the clinical consequences ensue.

Five types of defects have been established:

- In the most common, class 1, no immunoprecipitable (cross-reacting material (CRM)) receptor protein is found.
- In class 2, the protein cannot be transported to the endoplasmic reticulum and the Golgi complex.
- In class 3, the receptor does not properly bind LDL.
- In class 4, the receptor does not cluster in the coated pits and bound LDL is not internalized.
- In class 5, the receptors bind and internalize in coated pits but are unable to release the LDL in the endosome and recycle it.

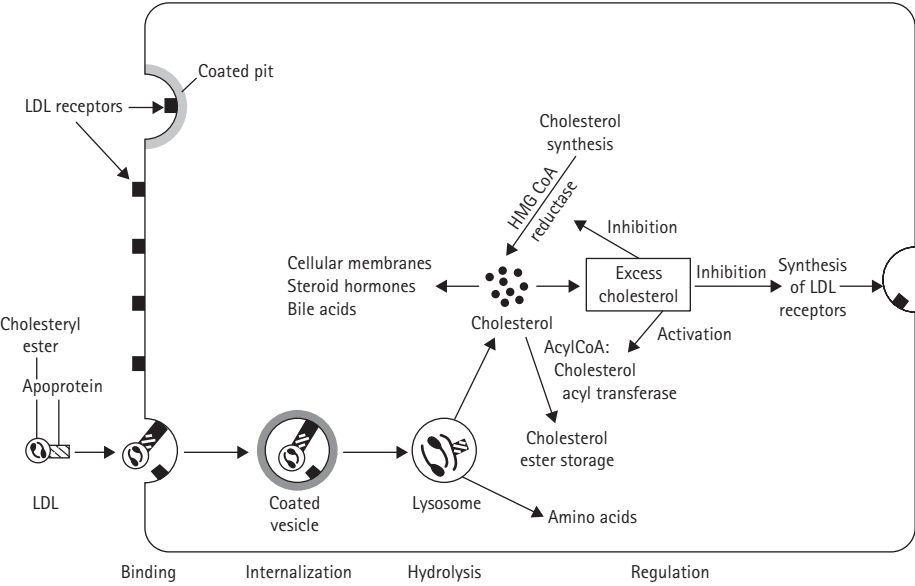


Figure 85.1 The low density lipoprotein (LDL) receptor and its role in the regulation of the metabolism of cholesterol.

The disease is caused by mutations in the *LDLR* gene. The gene has been mapped to the short arm of chromosome 19, at p13.1-13.3. A very large number and variety of mutations have been defined [8] and there are now two websites (www.ucl.ac.uk/fh/ and www.umd.necker.fr) [9–11]. The most common, class 1 mutations (Table 85.1) are null alleles in the sense that there is no immunoprecipitable receptor protein [12]. Mutations have been defined in each of the four classes. Homozygous individuals may have two identical mutant alleles, or they may be compound heterozygotes, who inherited a different allele from each parent.

CLINICAL ABNORMALITIES

Homozygous familial hypercholesterolemia

Hypercholesterolemia is present from birth in heterozygotes, as well as homozygotes, but homozygotes have severe hypercholesterolemic disease [13]. Their cholesterol concentrations range from 600 to 1200 mg/dL (15–30 μ mol/L) [14, 15]. The first clinical manifestation is usually the appearance of xanthomas (Figures 85.2, 85.3, 85.4, 85.5, 85.6, and 85.7).

Xanthomas may be flat (planar), tuberous, or tendinous.

Xanthomatous deposits are particularly common over the Achilles tendon and the extensor tendons of the hands. Tuberous xanthomas are seen over the elbows, knees, and elsewhere. Subperiosteal xanthomas may be seen below the knee at the tibial tuberosity and at the elbow. Trauma appears to influence the local occurrence of these lesions. Cutaneous xanthomas may be bright orange or yellow and they are prominent over the buttocks and the hands. The interdigital web between the first and second fingers is a favorite site. Xanthomas sometimes occur on the tongue or the buccal mucosa. An arcus about the cornea is regularly seen in homozygotes prior to the age of ten years (Figure 85.8).

The most significant clinical consequence of familial hypercholesterolemia is the occurrence of severe atherosclerosis in the aorta and coronary arteries because LDL-derived cholesterol is also deposited in arterial atheromatous plaques [16]. Peripheral and cerebral vessels are also involved. Plaques contain abundant deposits of lipid in the extracellular space and in large foamy cells. Disease of the heart tends to be rapidly progressive. Patients may have clinical angina as early as five years of age. Myocardial infarctions have been recorded as early as 18 months and three years of age. Most patients have died of this disease by 30 years of age [17].

Table 85.1 Classes of mutation of the low density lipoprotein (LDL) receptor

Class	Defect	Binding of low density lipoprotein	Internalization
1	Synthesis	Absent	–
2	Transport to Golgi	Absent or reduced	Normal
3	Binding of low density lipoprotein	Reduced or absent	Normal
4	Clustering in coated pits	Normal	Defective
5	Discharge in endosome (recycling)	Normal	Normal

There is evidence of genetically determined phenotypic variation in that homozygotes who have no LDL receptor function tend to have more relentless disease (with a mean age at death of 11 years) than those with defective receptors with some function (only one of 26 patients had died by the time of the report [18] and he was 23 years old). There is also evidence that other genes or factors modify the expression of the disease. In one family of two siblings with the identical mutation in the LDL receptor gene, one died at three years but the other was asymptomatic until 14 years [19]. In an effort to enhance the assessment of risk of vascular disease, a cholesterol-year score has been developed at the National Institutes of Health, similar to the pack-years score for cigarette smokers. By multiplying the number of years a patient has had a certain level of cholesterol (mg/dL), a score is achieved which in a series of 11 consecutive homozygous patients correlated very well with the development of angina [20].

Xanthomatous deposits are also found on the endocardium and on the mitral and aortic valves, leading to regurgitation and stenosis [21, 22]. The aortic valve hemodynamics may be indistinguishable from rheumatic or calcific aortic stenosis [2, 23, 24]. A diagnosis of acute rheumatic fever may also be suspected, on the basis of migratory painful joints and an elevated sedimentation rate, which patients may also have [25, 26]. Recurrent attacks of arthritis or tenosynovitis occur in the ankles, wrists, and proximal interphalangeal joints [22, 26, 27].



Figure 85.2 MAS: A 12-year-old Egyptian boy with homozygous familial hypercholesterolemia. In addition to multiple xanthomas, he had severe aortic stenosis and died in an attempt at surgical correction. He had a nine-year-old affected brother and the parents were first cousins.



Figure 85.3 MAS: Xanthomas over the elbow.



Figure 85.4 MAS: Cutaneous xanthomas over the knee.



Figure 85.5 MAS: Tendinous xanthomas were evident over each metacarpophalangeal joint, the distal interphalangeal joints of the first, second, and third fingers, and the proximal interphalangeal joints of the second and fifth fingers.



Figure 85.6 MAS: There were medial xanthomas of both feet.



Figure 85.7 BASJ: A seven-year-old with homozygous familial hypercholesterolemia. There were xanthomas over the Achilles tendon and the elbows, as well as the knees, from the age of three years.

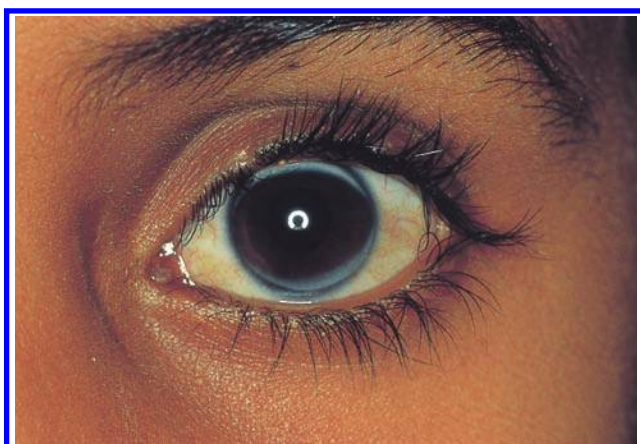


Figure 85.8 BASJ: At seven years, the arcus senilis was well developed.

These symptoms tend to last for 3 to 12 days and subside spontaneously. The elevated sedimentation rate may be present in the absence of arthritis, or absent when joint symptoms are present [26]. There may also be a two-fold elevation of the plasma concentration of fibrinogen.

Heterozygous familial hypercholesterolemia

In heterozygotes, concentrations of cholesterol in the blood range from 270 to 550 mg/dL (7–14 $\mu\text{mol/L}$ [28]; mean levels approximate 350 mg/dL (9 $\mu\text{mol/L}$) [13, 14, 29, 30]. Levels of triglycerides are usually normal [29]. Phospholipids are slightly elevated: they are more consistently elevated in homozygotes [31]. The increased cholesterol content of the plasma is entirely in the LDL fraction [3, 4, 32]. The normal concentration of LDL cholesterol is 110 ± 25 mg/dL. In heterozygotes, the mean levels reported [13] were 241 ± 60 , whereas in homozygotes the mean was 625 ± 160 mg/dL. Levels of high density lipoprotein (HDL) cholesterol tend to be a bit lower in both heterozygotes and homozygotes than in normals. In the absence of hypertriglyceridemia, an elevated level of cholesterol in the blood indicates that LDL cholesterol level is elevated.

Heterozygotes usually develop xanthomas by the time of death [29]. They occur typically over tendons such as the Achilles. Heterozygotes regularly develop xanthelasma, the palpebral xanthoma that is rarely seen in homozygotes. People with normal concentrations of cholesterol may also develop xanthelasma [3]. The corneal arcus is also seen in people with normal lipid metabolism. In heterozygotes, it is found in 10 percent by 30 years of age and in 50 percent of those over 30 years [32].

Clinical manifestations of coronary artery disease appear in heterozygotes as early as the fourth decade [33]. Its pattern is much more variable than in homozygotes. Mean age at death in males was 55 years and in females 64 years [34]. The probability of a coronary event was 16 percent by the age of 40 years and 52 percent by the age of 60 years in males [35]. In females, the probability was 33 percent by the age of 60 years.

GENETICS AND PATHOGENESIS

Familial hypercholesterolemia is classically caused by mutations in the gene for the low density lipoprotein receptor (LDLR) (Figure 85.1). It is also caused by mutations in the APOB and in the PCSK9 gene, the latter coding for the proprotein convertase subtilisin/kenin type 9. In 1358 French probands with autosomal dominant FH, mutations in LDLR were found in 1003 patients; of these mutations, 46 percent were missense, 13.6 percent frameshift, 11.3 percent nonsense, and 9.7 percent major rearrangements [36]. Mutations in the APOB gene were found in 6.6 percent of patients and the PCSK9 in 0.7

percent of patients. Finally, in 19.0 percent of the probands, no mutation was found [36]. Further evidence of genetic heterogeneity in FH was evidenced by a large French family in which mutations could be found in none of the three genes [37]. A genome-wide scan led to the discovery of another disease-causing gene named *HCHOLA4* which was located at chromosome 16q22. It was concluded that there are other FH-related genes because in nine families mutations were found in none of the four genes.

There are a number of allelic gene mutations in *LDLR*, the gene for the receptor [36–40] in a locus on the short arm of chromosome 19, which was placed at p13.1 to 13.3 [41]. Heterozygotes are found in a frequency of one in 500 [42]. This appears to be the most common single gene disease in humans, and it is seen throughout the world. It is expressed as dominant in heterozygotes. Homozygotes have two abnormal copies of the gene.

Cultured cells require cholesterol for survival. It is a necessary component of the plasma membrane of the cells. Amounts in excess of what is required are stored as cholesterol ester. Mammalian cells grown in serum utilize its LDL rather than synthesizing the compound [43]. The critical component in the uptake of cholesterol from LDL is the highly specific receptor that binds the apoprotein B-100 of the LDL [44–46]. Lipoproteins transport lipids to tissues following hydrolysis catalyzed by lipoprotein lipase (LPL) (Chapter 87); metabolism proceeds through intermediate metabolites, chylomicron remnants and very low density lipoprotein (VLDL) remnants. Both are rich in cholesterylesters and apoE, and are considered atherogenic lipoproteins, since they accumulate in the arterial walls [46]. An automated method for measuring remnant lipoprotein cholesterol has been shown to be fast and accurate and to be of use in monitoring situations such as postprandial increase in lipids and the metabolic syndrome [46].

LDL is a large, spherical particle with an oily core made up of many cholesterol molecules in ester linkage to fatty acids. There is a hydrophilic phospholipid coat in which one large protein (apoprotein B-100) is embedded. This apoprotein is recognized and bound by the receptor, which is an acidic glycoprotein. The receptor has been solubilized and purified to homogeneity. Its apparent molecular weight is 160 kDa [47–49]. It is synthesized as a precursor with an apparent molecular weight of 120 kDa and, 30 minutes after synthesis, it is converted to the apparently larger form and inserted into the plasma membrane.

LDL is taken up or internalized by a process termed receptor-mediated endocytosis [50–52]. The receptors are found in coated pits of the plasma membrane, where the surface is indented (Figure 85.1). Pits, containing bound LDL, invaginate and pinch off to form coated vesicles, which migrate to lysosomes and fuse with them. There, hydrolysis takes place to yield free cholesterol.

Intracellular cholesterol regulates its own intracellular concentration by means of an elegant system of feedback controls [53]. Some is required in the synthesis of cell surface membranes and in specialized cells, such as the adrenal and

liver, and some is converted to steroid hormones and bile acids. Excess may be stored as cholesterol ester, and the enzyme catalyzing this, acylCoA:cholesterol acyltransferase (ACAT), is activated by cholesterol [54]. The rate-limiting step in intracellular cholesterol synthesis is catalyzed by 3-hydroxy-3-methylglutaryl-CoA (HMG CoA) reductase. Regulation occurs at this level in that cholesterol suppresses the synthesis of this enzyme, thus turning off cholesterol biosynthesis [55]. The third regulatory process is a turning off by cholesterol of the synthesis of LDL receptors [56]. This prevents entry of additional LDL and the overloading of cells with cholesterol.

These considerations have relevance to the pathogenesis of atherosclerosis and coronary artery disease in normal individuals who do not have a defect in the LDL receptor. The consumption of a diet rich in dairy products, eggs, and animal meats provides enough cholesterol to overload the system and turn off the synthesis of LDL receptors. This protects the cell against too much cholesterol, but then excessive amounts of LDL accumulate in the blood and cholesterol is laid down in atherosclerotic plaques.

The LDL receptor is a cell-surface glycoprotein containing both N-linked and O-linked oligosaccharide chains [57]. The protein is synthesized in the endoplasmic reticulum and then migrates to the Golgi complex; in the process, the mannose-rich portion of the precursor protein is reduced and O-linked sugars, including sialic acid, are added to the core N-acetylgalactosamine [57]. Next, the receptor moves to the cell surface to cluster in the coated pits, which are lined by a surface protein, clathrin [58]. The coated pits invaginate to form endocytic vesicles, which fuse to form endosomes. The pH in the endosome falls, and LDL dissociates from the receptor which then returns to the surface ready to initiate another cycle of LDL binding and transport.

The gene contains 18 exons spanning 45 kb. The mRNA is 5.3 kb; it codes for a protein of 860 amino acids [59]. The very large number of mutations so far identified have fallen into five classes representing the five phenotypic groups [12, 57, 60–69]. Class 1 mutations are null alleles which produce no immunoprecipitable protein. At least one class 1 allele was found in over half of 128 fibroblast lines studied. Among 32 of these alleles studied, 12 had large deletions [12] recognizable on Southern blots. Four French-Canadian homozygotes were found to have a deletion over 10 kb involving the promoter and exon 1 [61]. A different 5 kb deletion of exons 13–15 leads to a truncated mRNA, but no protein [62]. In patients with two null alleles, there is no immunoprecipitable protein and receptors cannot be seen electron microscopically [12, 50].

In class 2 mutations, the proteins synthesized fail to be transported to the Golgi and the receptors accumulate intracellularly. These, too, are relatively common. Two of them were small deletions in exon 4. Deletion in exon 4 is the mutation for the Watanabe rabbit (WHHL), an animal model for familial hypercholesterolemia [63, 64]. These rabbits have less than 5 percent of the normal

number of LDL receptors, and high circulating levels of LDL and cholesterol, and they develop atherosclerosis and infarctions by two years of age. A nonsense mutation in which a single nucleotide substitution produces a stop codon that leads to a truncated protein has been called the Lebanese allele and has been found in several unrelated Arab patients [65]. A majority of class 2 mutants are leaky in the sense that some transport remains.

In class 3 mutations, the proteins are synthesized and they are transported normally, but their structure is altered so that they fail to bind the LDL properly. Levels of binding activity range from 2 to 30 percent of normal. Three mutations documented included two deletions and a duplication; the former do not bind LDL, indicating deletion in the ligand-binding domain, while the product of the duplication binds reduced quantities of LDL [57]. Differential effects on binding are illustrated by FH Paris-1 in which deletion of exon 5 leads to binding of VLDL, but not LDL [61], while FH French Canadian-3 binds neither [70].

Class 4 mutations are altered in their ability to cluster in the coated pits and consequently they fail to internalize bound LDL. These defects are rare, but interesting. Among mutations identified early, there were two deletions, an insertion, a nonsense mutation, and a missense mutation [66]. The stop codon in the nonsense mutation leaves only two of the normal 50 amino acids in the cytoplasmic domain. The insertion adds eight amino acids in this domain and the missense mutation changes a tyrosine to a cysteine at the 80th amino acid of this domain [67]. These observations suggested that this area is critical for binding to a protein as a requirement for movement into the clathrin-coated pits. This was the first evidence that clustering in the pits was required for transport into cells. The two deletions appear to constitute a different subclass of defects in which the membrane spacing domain is altered and the truncated receptors are largely secreted into the medium of cultured cells [66].

Class 5 mutations code for receptors that cannot discharge the ligand in the endosome and thus cannot recycle to the cell surface. The receptor is then degraded. Deletion of exons 7–14 in FH Osaka-2 leads to this phenotype [71]. A small number of mutations has been found in the promotor [72–74].

Exon-by-exon sequence analysis (EBESA) identified mutations in the *LDLR* gene; p.R3500W was found in eight probands of many Taiwanese families; of them, 25 were missense, five nonsense, and six frameshift mutations in 52; 11 probands were novel. Of the 42 probands with no mutations detected by EBESA, eight had abnormal multiple ligation-dependent probe amplification (MLPA) patterns, with six deletions. Mutations did not correlate well with lipid profiles or failure to lower LDL with statins [75]. A mutation G918>T of the *PCSK9* (p.R306S) exon 6 was reported in a Chinese family [76].

Double heterozygosity for mutations in the *LDLR* gene and the *APOB* gene have been reported [3]. A combination

of p.Leu479Pro in *LDLR* and p.Arg3527Gln was found in a 15-year-old girl. A double heterozygosity for *LDLR* and *PCSK9* has also been observed [77]. In general, these combinations lead to more severe disease. Two types of mutations should be considered in a family with an *LDLR* mutation and hypercholesterolemic relatives who do not carry the mutation.

Prenatal diagnosis of homozygous familial hypercholesterolemia has been made [78] by assay of LDL receptor activity in cultured amniocytes. In a series of pregnancies at risk, one was homozygous affected, two were heterozygotes, and one was normal. Prenatal diagnosis of an affected fetus has also been made by fetal blood sampling and analysis of cholesterol [79]. If the mutation is known, molecular diagnosis would be the procedure of choice. Heterozygosity for the Lebanese mutation has been documented in chorionic villus material [80].

Heterozygosity has been diagnosed in cord blood [13], as well as prenatally, but the assay of cord blood is not a reliable method of screening the general public. Even at the age of one year, when the methodology is more accurate, family study would be required to determine that an elevated level of LDL cholesterol is caused by familial hypercholesterolemia. Defective LDL receptor function can be documented in cultured fibroblasts or lymphocytes [79]. DNA-based diagnosis is feasible in populations in which a particular mutation is common. Patients with heterozygous hypercholesterolemia who inherited the gene from their mother had slight but significant increases of total cholesterol, LDL-C, and ApoB levels later in life than those who inherited the gene from their father, suggesting maternal programming during pregnancy [81].

TREATMENT

Homozygotes

Transplantation of the liver has been performed in a six-year-old child [82] with homozygous disease. The LDL receptors of the transplanted liver removed cholesterol from the plasma at a near normal rate and effectively reversed the abnormal concentrations. In another patient successfully transplanted, lesions in the coronary arteries regressed, as did xanthomas [20]. Experience with a small number of other patients confirmed dramatic fall in plasma LDL [83–85].

Homozygotes are generally resistant to the drugs and diet that are effective in heterozygotes, but those with some functional receptor activity may respond. The most practical and effective approach to the treatment of most homozygotes has been the removal of LDL by plasmapheresis or LDL apheresis [20, 86]. These procedures lower blood concentrations of cholesterol appreciably, and xanthomas have been observed to regress, as have lesions in the coronary arteries, which were limiting flow. Currently, this appears to be the treatment of choice [87, 88]. LDL

apheresis was found to decrease significantly levels of ferritin, transferrin, and vitamin B₁₂, and some patients became mildly anemic, but there was no change in plasma iron saturation or folic acid [89].

Lowering of cholesterol levels has also been reported following portacaval anastomosis [90].

Gene therapy has been undertaken in homozygous familial hypercholesterolemia [91]. In this *ex vivo* technique, hepatocytes were isolated from the patient and grown in culture, transfected with the normal gene, and reinjected into the portal circulation. The procedure was effective in lowering cholesterol in the WHHL rabbit and a human protocol was approved, but results were disappointing.

Germ-line interruptions in the *LDLR* and *Apobec1* genes in mice provide a model for homozygous FH. Introduction of the gene for mouse *LDLR* with an adenovirus vector to the livers of *LDLR*(-/-)*Apobec1*(-/-) mice led to an 87 percent regression of atherosclerotic lesions [92].

A porcine model of FH has been proposed to test the efficacy of drug eluting stents because it is thought to be a better model of intimal response, similar to that of man [93].

Heterozygotes

Two major approaches have been developed for the treatment of heterozygotes. These patients have one normal LDL receptor gene that is known to be under feedback control. The first group of drugs to be employed is anion-binding resins such as cholestyramine and colestipol [94]. They prevent the recycling of bile acids and thus stimulate the synthesis of LDL receptors and lower LDL cholesterol concentrations by 15–20 percent. This was enough to reduce the incidence of myocardial infarctions by 20 percent in a ten-year prospective study [38]. This approach is limited by the fact that the cell also responds to cholesterol deprivation by induction of HMG CoA reductase synthesis and by increasing *de novo* synthesis of cholesterol, which inhibits the synthesis of new LDL receptors.

The development of drugs that inhibit cholesterol biosynthesis by inhibiting HMG CoA reductase has provided a systematic approach to treatment by combination with bile acid sequestration. The first of these drugs was mevastatin (Compactin), a compound isolated from *Penicillium* [95] that has a side chain resembling mevalonic acid. A more potent inhibitor isolated from *Aspergillus* differs in structure only in the substitution of a methyl for hydrogen group and is called mevinolin or lovastatin [96]. Both compounds lower blood levels of LDL and cholesterol and significantly increase LDL receptors. Combined therapy with cholestyramine and mevinolin lowers LDL cholesterol levels by 50–60 percent [97]. Currently, lovastatin and chemically modified natural statins (pravastatin and simvastatin) or synthetic statins (fluvastatin, cerivastatin, and atorvastatin) are in use in human therapy, not only for FH heterozygotes, but for many others with hypercholesterolemia [98]. These drugs

lower LDL cholesterol. They are not without side effects, including hepatic toxicity and myopathy, which may manifest as rhabdomyolysis. The addition of nicotinic acid to the regimen may further improve the effect on levels of cholesterol [97].

In a study in Japan, the age at onset of coronary artery disease was 58.8 ± 12.5 years in 25 patients treated with statins at the onset of the study, which was significantly higher than that of the 76 patients not treated (47.6 ± 10.5 years) ($p < 0.001$). The average age of onset of coronary disease was significantly higher after widespread use of statins in Japan [99]. In heterozygous FH, statin therapy was found to yield a decrease in LDL cholesterol and a slower increase in carotid intimal/medial thickness [100].

In children with familial hypercholesterolemia, diet has been the predominant mode of treatment. Anion exchange resins, such as cholestyramine and colestipol may be effective, but they are unpalatable and poorly tolerated. In a search of statin trials reviewed from Medline, eight randomized, double-blind, controlled studies were found involving 897 patients less than 18 years of age. Therapy with statins was found to reduce serum LDL cholesterol levels by 23 to 40 percent. Safety was evident in no differences in transaminases or creatine kinase, but long-term safety remains to be determined [101]. Pediatric patients with FH treated with antihyperlipidemic agents not only have decreased levels of serum lipid [75], but also of plasma von Willebrand factor antigen which might further impede the development of the atherosclerosis [102].

In an approach to complications of statin therapy, rosuvastatin administration to children with heterozygous FH was found to cause a significant decrease in peripheral blood mononuclear cell CoQ10 concentrations [103].

A novel compound, ezetimibe, that selectively inhibits cholesterol uptake, when used in addition to statins was found to have an additional LDL cholesterol-lowering effect. There was also a progressive decrease in carotid intima-media thickness [104].

Mipomersen, an oligonucleotide antisense inhibitor directed against apoB mRNA, has been studied in patients with heterozygous FH also receiving conventional lipid-lowering therapy. Significant reduction of LDL cholesterol of 21 and 34 percent were encountered in a dose-related fashion. Levels of APOB were also reduced [105].

All medications which lower lipids are contraindicated during pregnancy [106]. A prudent diet for these patients is one low in cholesterol and saturated fat. Oxidative stress contributes to lipid peroxidation and decreases nitric oxide (NO) bioavailability in atherosclerosis. Long-chain (n-3) polyunsaturated fatty acids (PUFA) are easily oxidized and improve endothelial function. In experimental animals, a fish oil-rich diet increased NO production and endothelial NO synthase expression. A fish oil-rich or supplemented diet appears prudent [107]. Double heterozygotes for mutations in *LDLR* and *APOB* tend to respond to treatment with statins [3].

REFERENCES

1. Brown MS, Goldstein JL. Familial hypercholesterolemia: model for genetic receptor disease. *Harvey Lect Ser* 1979; **73**: 163.
2. Khachadurian AK. The inheritance of essential familial hypercholesterolemia. *Am J Med* 1964; **37**: 402.
3. Taylor A, Bayly G, Patel K *et al*. A double heterozygote for familial hypercholesterolemia and familial defective apolipoprotein B-100. *Ann Clin Biochem* 2010; **47**: 487.
4. Dai YF, Sun LY, Zhang XB *et al*. Research progression of LDLR mutations in chinese familial hypercholesterolemia. *Yi Chuan* 2011; **33**: 1.
5. Gofman JW, Rubin L, McGinley JP, Jones HB. Hyperlipoproteinemia. *Am J Med* 1954; **17**: 514.
6. Frederickson DS, Levy RI, Lees RS. Fat transport in lipoproteins: an integrated approach to mechanisms and disorders. *N Engl J Med* 1967; **276**: 34, 94, 148, 215, 273.
7. Goldstein JL, Brown MS. Binding and degradation of low density lipoprotein by cultured human fibroblasts: comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *J Biol Chem* 1974; **249**: 5153.
8. Day INM, Whittall RA, O'Dell SD *et al*. Spectrum of LDL receptor gene mutations in heterozygous familial hypercholesterolemia. *Hum Mutat* 1997; **10**: 116.
9. Wilson DJ, Gahan M, Haddad L *et al*. A world wide web site for low-density lipoprotein receptor gene mutations in familial hypercholesterolemia: Sequence-based tabular and direct submission data handling. *Am J Cardiol* 1998; **81**: 1509.
10. Varret M, Rabes J-P, Collod-Beroud G *et al*. Software and database for the analysis of mutations in the human LDL receptor gene. *Nucleic Acids Res* 1997; **25**: 172.
11. Varret M, Rabes J-P, Thiar R *et al*. LDLR database (second edition): new additions to the database and the software and results of the first molecular analysis. *Nucleic Acids Res* 1998; **26**: 248.
12. Hobbs HH, Lettersdorf E, Goldstein JL *et al*. Multiple crm-mutations in familial hypercholesterolemia: evidence for 13 alleles including four deletions. *J Clin Invest* 1988; **81**: 909.
13. Kwiterovich PO Jr, Levy RI, Frederickson DS. Neonatal diagnosis of familial type II hyperlipoproteinaemia. *Lancet* 1973; **1**: 118.
14. Kwiterovich PO Jr, Frederickson DS, Levy RI. Familial hypercholesterolemia (one form of familial type II hyperlipoproteinemia). A study of its biochemical genetic and clinical presentation in childhood. *J Clin Invest* 1974; **53**: 1237.
15. Khachadurian AK. A general view of clinical and laboratory features of familial hypercholesterolemia (type II hyperbetalipoproteinemia). *Protides Biological Fluids* 1971; **19**: 315.
16. Buja LM, Kovanen PT, Bilheimer DW. Cellular pathology of homozygous familial hypercholesterolemia. *Am J Pathol* 1979; **97**: 327.
17. Stefel HC, Baker SG, Sandler MP *et al*. A host of hypercholesterolemia homozygotes in South Africa. *Br Med J* 1980; **281**: 633.
18. Goldstein JL, Brown MS. The LDL receptor defect in familial hypercholesterolemia: implications for pathogenesis and therapy. *Med Clin North Am* 1982; **66**: 335.
19. Hobbs HH, Brown MS, Russell DW *et al*. Deletion in LDL receptor gene occurs in majority of French Canadians with familial hypercholesterolemia. *N Engl J Med* 1987; **317**: 734.
20. Hoeg JM. Familial hypercholesterolemia. What the zebra can teach us about the horse. *J Am Med Assoc* 1994; **271**: 543.
21. Maher JA, Epstein FH, Hand EA. Xanthomatosis and coronary heart disease: necropsy study of two affected siblings. *Arch Intern Med* 1958; **102**: 137.
22. Schettler FC. Essential familial hypercholesterolemia. In: Schettler FG, Blyd GS (eds). *Atherosclerosis*. Amsterdam: Elsevier, 1969: 543.
23. Haitas B, Baker SG, Meyer TE *et al*. Natural history and cardiac manifestations of homozygous familial hypercholesterolaemia. *Q J Med* 1990; **76**: 731.
24. Beppu S, Minura Y, Sakakibara H *et al*. Supravalvular aortic stenosis and coronary ostial stenosis in familial hypercholesterolemia: two-dimensional echocardiographic assessment. *Circulation* 1983; **67**: 878.
25. Khachadurian AK. Migratory polyarthritis in familial hypercholesterolemia (type II hyperlipoproteinemia). *Arthritis Rheum* 1968; **11**: 385.
26. Khachadurian AK. Persistent elevation of the erythrocyte sedimentation rate (ESR) in familial hypercholesterolemia. *J Med Liban* 1967; **20**: 31.
27. Rooney PJ, Third J, Madkour MM *et al*. Transient polyarthritis associated with familial hyperbetalipoproteinemia. *Q J Med* 1978; **47**: 249.
28. Brown MS, Goldstein JL. Familial hypercholesterolemia: genetic biochemical and pathophysiologic considerations. *Adv Intern Med* 1975; **20**: 273.
29. Schrott HG, Goldstein JL, Hazzard WR *et al*. Familial hypercholesterolemia in a large kindred. Evidence for a monogenic mechanism. *Ann Intern Med* 1972; **76**: 711.
30. Nevin NC, Slack H. Hyperlipidaemic xanthomatosis. II. Mode of inheritance in 55 families with essential hyperlipidaemia and xanthomatosis. *J Med Genet* 1968; **5**: 9.
31. Slack J, Mills GL. Anomalous low density lipoproteins in familial hyperbetalipoproteinaemia. *Clin Chim Acta* 1970; **29**: 15.
32. Frederickson DS, Levy RI. Familial hyperlipoproteinemia. In: Stanbury JB, Wyngaarden JB, Frederickson DS (eds). *The Metabolic Basis of Inherited Disease*, 3rd edn. New York: McGraw-Hill, 1972: 545.
33. Slack J. Risks of ischaemic heart-disease in familial hyperlipoproteinemia states. *Lancet* 1969; **2**: 1380.
34. Heiberg A. The risk of atherosclerotic vascular disease in subjects with xanthomatosis. *Acta Med Scand* 1975; **198**: 249.
35. Stone NJ, Levy RI, Frederickson DS, Veter J. Coronary artery disease in 116 kindred with familial type II hyperlipoproteinemia. *Circulation* 1974; **49**: 476.
36. Marduel M, Carrie A, Sassolas A *et al*. Molecular spectrum of autosomal dominant hypercholesterolemia in France. *Hum Mutat* 2010; **31**: 1811.

37. Marques-Pinheiro A, Marduel M, Rabes JP *et al.* A fourth locus for autosomal dominant hypercholesterolemia maps at 16q22.1. *Eur J Hum Genet* 2010; **18**: 1236.
38. Brown MS, Goldstein JL. How LDL receptors influence cholesterol and atherosclerosis. *Sci Am* 1984; **251**: 58.
39. Brown MS, Goldstein JL. Receptor-mediated endocytosis: insights from the lipoprotein receptor system. *Proc Natl Acad Sci USA* 1979; **76**: 3330.
40. Brown MS, Goldstein JL. Receptor-mediated control of cholesterol metabolism. *Science* 1976; **181**: 150.
41. Lindgren V, Luskey KL, Russell DW, Francke U. Human genes involved in cholesterol metabolism: chromosomal mapping of the loci for the low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl-coenzyme A reductase with cDNA probes. *Proc Natl Acad Sci USA* 1985; **82**: 8567.
42. Slack J. Inheritance of familial hypercholesterolemia. *Atherosclerosis Rev* 1979; **5**: 35.
43. Goldstein JL, Brown MS. The LDL pathway in human fibroblasts: a receptor-mediated mechanism for the regulation of cholesterol metabolism. *Curr Top Cell Regul* 1976; **11**: 147.
44. Brown MS, Goldstein JL. Familial hypercholesterolemia: defective binding of lipoproteins to cultured fibroblasts associated with impaired regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. *Proc Natl Acad Sci USA* 1974; **71**: 788.
45. Mahley RW, Innerarity TL, Pitas RE *et al.* Inhibition of lipoprotein binding to cell surface receptors of fibroblasts following selective modification of arginyl residues in arginine-rich and B-apoproteins. *J Biol Chem* 1977; **252**: 7279.
46. Yamamura T, Ishigami M. Cutting-edge research on the metabolism of remnant lipoproteins. *Rinsho Byori* 2010; **58**: 613.
47. Schneider WJ, Basu SK, McPhaul MJ *et al.* Solubilization of the low density lipoprotein receptor. *Proc Natl Acad Sci USA* 1979; **76**: 5577.
48. Schneider WJ, Goldstein JL, Brown MS. Partial purification and characterization of the low density lipoprotein receptor from bovine adrenal cortex. *J Biol Chem* 1980; **255**: 11442.
49. Schneider WJ, Beisiegel U, Goldstein JL, Brown MS. Purification of the low density lipoprotein receptor an acidic glycoprotein of 164,000 molecular weight. *J Biol Chem* 1982; **257**: 2664.
50. Anderson RGW, Goldstein JL, Brown MS. Localization of low density lipoprotein receptors on plasma membrane of normal human fibroblasts and their absence in cells from a familial hypercholesterolemia homozygote. *Proc Natl Acad Sci USA* 1976; **73**: 2434.
51. Anderson RGW, Brown MS, Goldstein JL. Role of the coated endocytic vesicle in the uptake of receptor-bound low density lipoprotein in human fibroblasts. *Cell* 1977; **10**: 351.
52. Orci L, Carpenter J-L, Perrelet A *et al.* Occurrence of low density lipoprotein receptors within large pits on the surface of human fibroblasts as demonstrated by freeze-etching. *Exp Cell Res* 1978; **113**: 1.
53. Brown MS, Goldstein JL. A general scheme for the regulation of cholesterol metabolism in mammalian cells. In: Dietschy JM, Gotto AM, Ontko J (eds). *Disturbances in Lipid and Lipoprotein Metabolism*. Bethesda, MD: American Physiological Society, 1978: 173.
54. Goldstein JL, Dana SE, Brown MS. Esterification of low density lipoprotein in human fibroblasts and its absence in homozygous familial hypercholesterolemia. *Proc Natl Acad Sci USA* 1974; **71**: 4288.
55. Brown MS, Dana SE, Goldstein JL. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured human fibroblasts: comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *J Biol Chem* 1974; **249**: 789.
56. Brown MS, Goldstein JL. Regulation of the activity of the low density lipoprotein receptor in human fibroblasts. *Cell* 1975; **6**: 307.
57. Tolleshaug H, Goldstein JL, Schneider WJ *et al.* Post-translational processing of the LDL receptor and its genetic disruption in familial hypercholesterolemia. *Cell* 1982; **30**: 715.
58. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science* 1986; **232**: 34.
59. Yamamoto T, Davis CG, Brown MS *et al.* The human LDL receptor: A cysteine-rich protein with multiple Alu sequences in its Mrna. *Cell* 1984; **39**: 27.
60. Hobbs H, Brown MS, Goldstein JL. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. *Hum Mutat* 1992; **1**: 445.
61. Hobbs HH, Brown MS, Goldstein JL, Russell DW. Deletion of exon encoding cysteine-rich repeat of LDL receptor alters its binding specificity in a subject with familial hypercholesterolemia. *J Biol Chem* 1986; **261**: 13114.
62. Lehrman MA, Russell DW, Goldstein JL, Brown MS. Exon-Alu recombination deletes 5 kilobases from low density lipoprotein receptor gene producing null phenotype in familial hypercholesterolemia. *Proc Natl Acad Sci USA* 1986; **83**: 3679.
63. Watanabe Y. Serial inbreeding of rabbits with hereditary hyperlipidemia (WHHL-Rabbit). Incidence and development of atherosclerosis and xanthoma. *Atherosclerosis* 1980; **36**: 261.
64. Yamamoto T, Bishop RW, Brown MS *et al.* Deletion in cysteine-rich region of LDL receptor impedes transport to cell surface in WHHL rabbit. *Science* 1986; **232**: 1230.
65. Lehrman MA, Schneider WJ, Brown MS *et al.* The Lebanese allele at the LDL receptor locus: nonsense mutation produces truncated receptor that is retained in endoplasmic reticulum. *J Biol Chem* 1987; **262**: 401.
66. Lehrman MA, Russell DW, Goldstein JL, Brown MS. Alu-Alu recombination deletes splice acceptor sites and produces secreted LDL receptor in a subject with familial hypercholesterolemia. *J Biol Chem* 1987; **262**: 3345.
67. Davis CG, Lehrman MA, Russell DW *et al.* The JD mutation in familial hypercholesterolemia: substitution of cysteine for tyrosine in cytoplasmic domain impedes internalization of LDL receptors. *Cell* 1986; **45**: 15.
68. Kajinami K, Mabuchi H, Inazu A *et al.* Novel gene mutations at the low density lipoprotein receptor locus: FH-Kanazawa and FH-Okayama. *J Intern Med* 1990; **227**: 247.
69. Day INM, Whittall RA, O'Dell SD *et al.* Spectrum of LDL receptor gene mutations in heterozygous familial hypercholesterolemia. *Hum Mutat* 1997; **10**: 116.

70. Leitersdorf E, Tobin EJ, Davignon J, Hobbs HH. Common low-density lipoprotein receptor mutations in the French Canadian population. *J Clin Invest* 1990; **85**: 1014.
71. Miyake Y, Tajima S, Funahashi T, Yamamoto A. Analysis of a recycling-impaired mutant of low density lipoprotein receptor in familial hypercholesterolemia. *J Biol Chem* 1989; **264**: 16584.
72. Jensen LG, Jensen HK, Nissen H *et al*. An LDL receptor promoter mutation in a heterozygous FH patient with dramatically skewed ratio between the two allelic mRNA variants. *Hum Mutat* 1996; **7**: 82.
73. Koivisto U-M, Palvimäki JJ, Janne OA, Kontula K. A single-base substitution in the proximal Sp1 site of the human low density lipoprotein receptor promoter as a cause of heterozygous familial hypercholesterolemia. *Proc Natl Acad Sci USA* 1994; **91**: 10526.
74. Sun X-M, Neuwirth C, Wade DP *et al*. A mutation (T-45 C) in the promoter region of the low-density-lipoprotein (LDL)-receptor gene is associated with a mild clinical phenotype in a patient with heterozygous familial hypercholesterolaemia (FH). *Hum Mol Genet* 1995; **4**: 2125.
75. Chiou KR, Charng MJ. Detection of mutations and large rearrangements of the low-density lipoprotein receptor gene in Taiwanese patients with familial hypercholesterolemia. *Am J Cardiol* 2010; **105**: 1752.
76. Lin J, Wang LY, Liu S *et al*. A novel mutation in proprotein convertase subtilisin/kexin type 9 gene leads to familial hypercholesterolemia in a Chinese family. *Chin Med J* 2010; **123**: 1133.
77. Pisciotta L, Priore OC, Cefalu AB *et al*. Additive effect mutations in LDLR and PCSK9 genes on the phenotype of familial hypercholesterolemia. *Atherosclerosis* 2006; **186**: 433.
78. Brown MS, Kovanen PT, Goldstein JL *et al*. Prenatal diagnosis of homozygous familial hypercholesterolaemia: expression of a genetic receptor disease *in utero*. *Lancet* 1978; **1**: 526.
79. De Gennes JL, Daffos F, Dairou F *et al*. Direct fetal blood examination for prenatal diagnosis of homozygous familial hypercholesterolemia. *Arteriosclerosis* 1985; **5**: 440.
80. Reshef A, Meiner V, Dann EJ *et al*. Prenatal diagnosis of familial hypercholesterolemia caused by the 'Lebanese' mutation at the low density lipoprotein receptor locus. *Hum Genet* 1992; **89**: 237.
81. Van der Graaf A, Vissers MN, Gaudet D *et al*. Dyslipidemia of mothers with familial hypercholesterolemia deteriorates lipids in adult offspring. *Arterioscler Thromb Vasc Biol* 2010; **12**: 2673.
82. Bilheimer DW, Goldstein JL, Grundy SM *et al*. Liver transplantation to provide low-density-lipoprotein receptors and lower plasma cholesterol in a child with homozygous familial hypercholesterolemia. *N Engl J Med* 1984; **311**: 1658.
83. Valdivielso P, Escolar JL, Cuervas-Mons V *et al*. Lipids and lipoprotein changes after heart and liver transplantation in a patient with homozygous familial hypercholesterolemia. *Ann Intern Med* 1988; **108**: 204.
84. Barbir M, Khaghani A, Kehely A *et al*. Normalisation of lipoproteins including Lp(a) after liver-heart transplantation in homozygous familial hypercholesterolaemia. *Q J Med* 1992; **85**: 807.
85. Téllez de Peralta G, Burgos Lázaro R. Transplantes multiorgánicos. *Rev Esp Cardiol* 1995; **48**: 46.
86. Thompson GR, Lowenthal R, Myant NB. Plasma exchange in the management of homozygous familial hypercholesterolaemia. *Lancet* 1975; **1**: 1208.
87. Gordon BR, Kelsey SF, Dau PC *et al*. Long-term effects of low-density lipoprotein apheresis using an automated dextran sulfate cellulose adsorption system. *Am J Cardiol* 1998; **81**: 407.
88. Tatami R, Inoue N, Itoh H *et al*. Regression of coronary atherosclerosis by combined LDL-apheresis and lipid-lowering drug therapy in patients with familial hypercholesterolemia: a multicenter study. *Atherosclerosis* 1992; **95**: 1.
89. Bramlage CP, Armstrong VW, Zapf A *et al*. Low-density lipoprotein apheresis decreases ferritin, transferrin and vitamin B₁₂, which may cause anemia in serially treated patients. *Ther Apher Dial* 2010; **14**: 136.
90. Bilheimer DW, Stone NJ, Grundy SM. Metabolic studies in familial hypercholesterolaemia: evidence for a gene-dosage effect *in vivo*. *J Clin Invest* 1975; **64**: 1420.
91. Wilson JM, Chowdury JR. Prospects for gene therapy of familial hypercholesterolemia. *Mol Biol Med* 1990; **7**: 223.
92. Kassim SH, Li H, Vandenberghe LH *et al*. Gene therapy in a humanized mouse model of familial hypercholesterolemia leads to marked regression of atherosclerosis. *PLoS One* 2010; **5**: e13424.
93. Tellez A, Krueger CG, Seifert P *et al*. Coronary bare metal stent implantation in homozygous LDL receptor deficient swine induces a neointimal formation pattern similar to humans. *Atherosclerosis* 2010; **213**: 518.
94. Grundy SM. Treatment of hypercholesterolemia by interference with bile acid metabolism. *Arch Intern Med* 1972; **130**: 638.
95. Endo A, Kuroda M, Tanzawa K. Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236 A and ML-236 B fungal metabolites, having hypocholesterolemic activity. *FEBS Lett* 1976; **72**: 323.
96. Alberts AW, MacDonald JS, Till AE, Tobert JA. Lovastatin. *Cardiovasc Drug Rev* 1989; **7**: 89.
97. Hoeg JM, Maher MB, Zech LA *et al*. Effectiveness of mevinolin on plasma lipoprotein concentrations type II hyperlipoproteinemia. *Am J Cardiol* 1986; **57**: 933.
98. Malloy MJ, Kane JP, Kunitake ST, Tun P. Complementarity of colestipol, niacin and lovastatin in treatment of severe familial hypercholesterolemia. *Ann Intern Med* 1987; **107**: 616.
99. Harada-Shiba M, Sugisawa T, Makino H *et al*. Impact of statin treatment on the clinical fate of heterozygous familial hypercholesterolemia. *J Atheroscler Thromb* 2010; **17**: 667.
100. Vergeer M, Zhou R, Bots ML *et al*. Carotid atherosclerosis progression in familial hypercholesterolemia patients: a pooled analysis of the ASAP, ENHANCE, RADIANCE 1, and CAPTIVATE studies. *Circ Cardiovasc Imaging* 2010; **3**: 398.
101. Vuorio A, Kuoppala J, Kovanen PT *et al*. Statins for children with familial hypercholesterolemia. *Cochrane Database Syst Rev* 2010; **(7)**: CD006401.
102. Yalçın SS, Güneş B, Unal S *et al*. Antihyperlipidemic agents cause a decrease in von Willebrand factor levels in pediatric patients with familial hyperlipidemia. *J Pediatr Endocrinol Metab* 2010; **23**: 765.

103. Avis HJ, Hargreaves IP, Ruiter JP *et al.* Rosuvastatin lowers coenzyme Q10 levels, but not mitochondrial adenosine triphosphate synthesis, in children with familial hypercholesterolemia. *J Pediatr* 2011; **158**: 458.
104. Avellone G, Di Garbo V, Guarnotta V *et al.* Efficacy and safety of long-term ezetimibe/simvastatin treatment in patients with familial hypercholesterolemia. *Int Angiol* 2010; **29**: 514.
105. Akdim F, Visser ME, Tribble DL *et al.* Effect of mipomersen, an apolipoprotein B synthesis inhibitor, on low-density lipoprotein cholesterol in patients with familial hypercholesterolemia. *Am J Cardiol* 2010; **105**: 1413.
106. Kusters DM, Homsma SJ, Hutten BA *et al.* Dilemmas in treatment of women with familial hypercholesterolaemia during pregnancy. *Neth J Med* 2010; **68**: 299.
107. Casós K, Zaragoza MC, Zarkovic N *et al.* A fish-oil-rich diet reduces vascular oxidative stress in apoE(–/–) mice. *Free Radic Res* 2010; **44**: 821.

Mevalonic aciduria

Introduction	642	Treatment	646
Clinical abnormalities	643	References	646
Genetics and pathogenesis	645		

MAJOR PHENOTYPIC EXPRESSION

Failure to thrive; diarrhea and malabsorption; psychomotor impairment; dysmorphic features, cataracts; retinal dystrophy; hypotonia; hepatosplenomegaly and lymphadenopathy; ataxia; recurrent crises of fever, arthralgia and skin eruption; hyperimmunoglobulin D (IgD); mevalonic aciduria; and defective activity of mevalonic acid kinase.

INTRODUCTION

Mevalonic aciduria was discovered in 1986, the first inborn error in the biosynthesis of cholesterol and nonsterol isoprenoid compounds [1]. It results from a deficiency of the activity of mevalonate kinase (Figures 86.1 and 86.2). The disorder is recognized by organic acid analysis of the urine via gas chromatography/mass spectrometry (GCMS). This compound can easily be missed in GCMS analysis [2].

Mevalonic acid (Figure 86.3) is 3-hydroxy-3-methyl-5-hydroxypentanoic acid. The compound spontaneously cyclizes to form the lactone under the acidic conditions usually employed in liquid partition chromatography, extraction, and other forms of preparation for organic acid analysis. The lactone does not open up under conditions of formation of trimethylsilyl (TMS) derivatives. The mono-TMS derivative of the lactone is formed under acid conditions and the tri-TMS derivative of mevalonic acid is formed after treatment with alkali. Methylation of the acid followed by formation of the TMS derivative provides the best approach to identification. The development of a

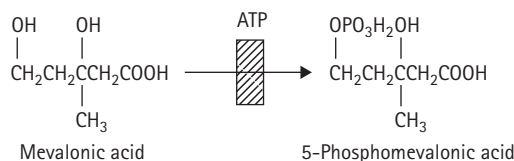


Figure 86.1 Mevalonate kinase, the site of the defect in mevalonic aciduria.

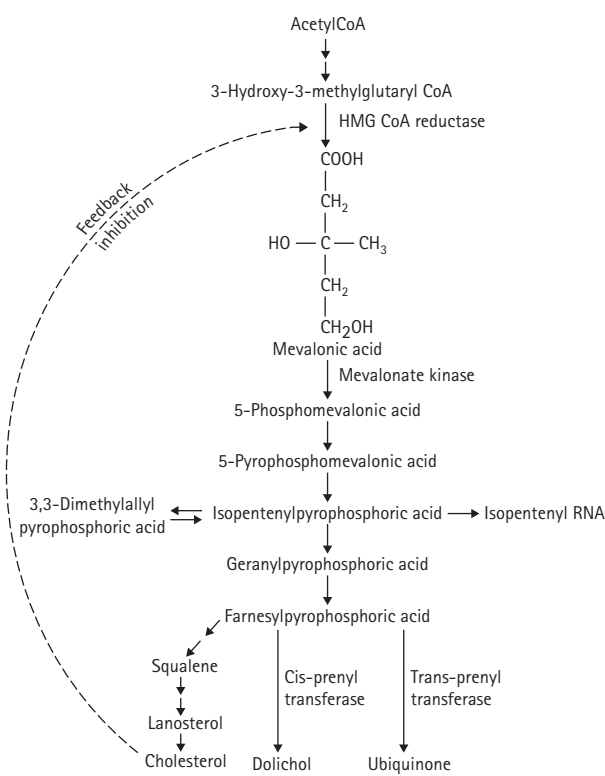


Figure 86.2 Metabolic pathways involving the formation of mevalonic acid and its role in the synthesis of cholesterol, dolichol and ubiquinone.

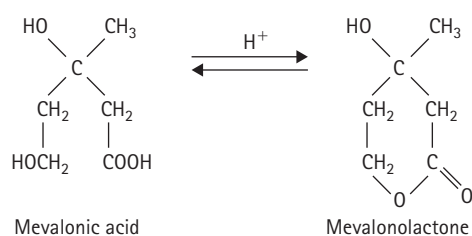


Figure 86.3 Structure of mevalonic acid and its lactone.

stable isotope dilution GCMS assay for mevalonic acid has facilitated quantification of the compound in body fluids [2].

In the mevalonate kinase reaction (Figures 86.1 and 86.2) the 5-hydroxy group of 3-R-mevalonic acid is phosphorylated to yield mevalonic-5-phosphate. The cDNA for the human enzyme has been cloned and localized to chromosome 12q24 [3]. At least 63 mutations have been defined [3–5], and genotype–phenotype correlations are emerging. Most mutations appear to affect stability or folding of the enzyme rather than its catalytic properties [5].

CLINICAL ABNORMALITIES

Only 20 patients have been reported [6, 7], but varying degrees of clinical severity have been observed, and the spectrum of phenotypes has been enlarged [8–11]. The most severely affected have died in infancy [1, 8] or childhood [9]. Less severely impaired patients have had developmental delay, ataxia, and hypotonia. Most have had recurrent crises of fever, tender lymphadenopathy, increase in liver and spleen size, arthralgia, and a morbilliform eruption. Acute phase reactants, the erythrocyte sedimentation rate, C-reactive protein and leukocytosis, as well as creatine kinase (CK) and transaminases, are elevated. It has recently been recognized that patients with the hyperimmunoglobulin



Figure 86.4 ZW: A 22-month-old with mevalonic aciduria. He was tiny and had virtually no subcutaneous fat. The penis was very small.

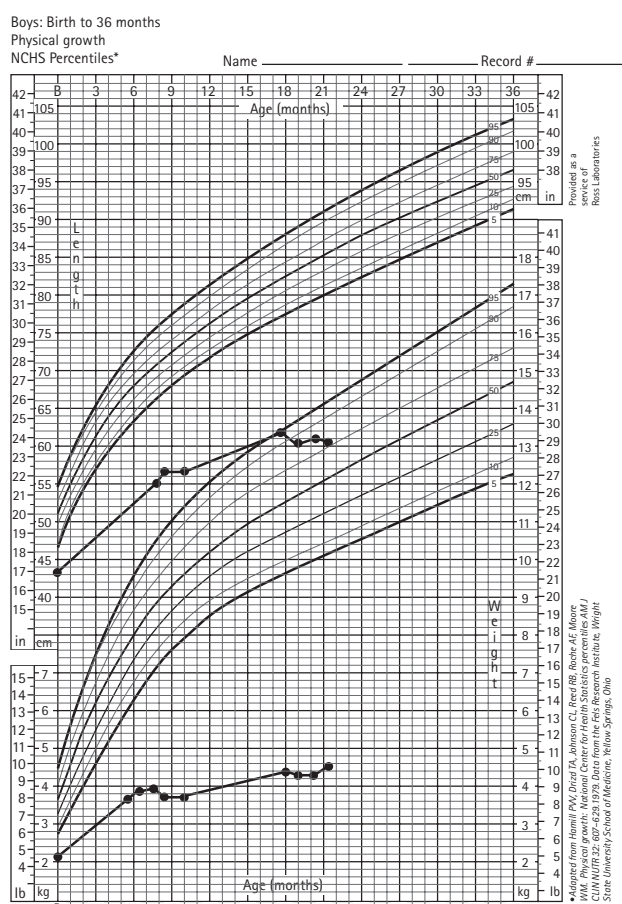


Figure 86.5 Anthropometric data on ZW illustrate the extreme failure to thrive.

D and periodic fever syndrome (HIDS) have mevalonic aciduria [12, 13]. Mutations in the mevalonate kinase gene have been found in patients with HIDS indicating that HIDS is a milder, allelic form of mevalonic aciduria [14, 15]. An even more attenuated presentation was observed in a five-year-old with cerebellar ataxia and retinal dystrophy and no febrile crises or hyperimmunoglobulin D elevation [7].

Our first patient [1] presented with severe failure to thrive, diarrhea, and hepatosplenomegaly, a picture that led to referral to gastroenterologists. At 19 months his weight, height, and head circumference were 4–8 SD below the mean for age. He had little or no subcutaneous fat (Figures 86.4 and 86.5). He died at 21 months [6]. Failure to thrive was present in nearly all of the early reported patients: it was described as severe in two [1, 16], moderate in five, and mild in two [6]. The two most severely affected had gastrointestinal symptoms suggesting intolerance to cows' milk. Hepatosplenomegaly was notable in five patients.

Psychomotor impairment has been characteristic of each of the early patients described. It was of such severity in the most severely affected patients that no social interaction was possible [1, 6, 8, 9]. Two siblings [16] had IQs of 60 and 65; in two others [6] IQs were 77 and 82, and

in the least severe patient [17, 18] the IQ was 85. Intellectual impairment has appeared to be nonprogressive. In contrast, ataxia and dysarthria developed after the second year of life in a majority of the patients surviving that long and became progressively more prominent. Imaging of the central nervous system revealed progressive cerebellar atrophy [7]. Deep tendon reflexes may be accentuated, and a crossed response may be elicited. There may be cortical thumbs and incomplete extension at the elbows and knees.

Hypotonia is observed regularly, and some patients have appeared to have myopathy. In one of the least severely affected patients [17], the complete picture was of static myopathy, borderline impaired mental development, and severe ataxia in a 12-year-old. Myopathy became more severe during febrile crises. One patient developed cardiomyopathy and heart block and required artificial ventilation for 2 weeks. Two patients had febrile convulsions at one and two years of age.

Cataracts were observed in a number of patients [11, 16]. Two others [6] developed uveitis and retinitis pigmentosa, which became worse with crises. Retinal dystrophy may take the form of bone-spicule retinitis pigmentosa or may be more subtle, thinned vessels and uneven retinal surface and abnormal electroretinogram, and there may be optic atrophy [7].

Dysmorphic features were described in all but a few patients [7, 17], but were described as subtle in four [6]. The characteristic picture (Figures 86.6 and 86.7) is of dolichocephaly with frontal bossing, posteriorly rotated low-set ears, antimongoloid slanting of the eyes, a small



Figure 86.6 Close-up of the face revealed the prominent forehead and the low-set ears, as well as the long philtrum and thin lips.

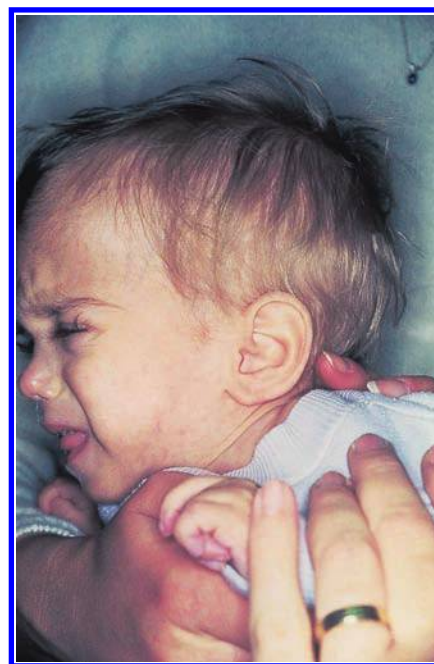


Figure 86.7 Profile illustrates the dolichocephaly and mild hypognathia. This ear was larger than the other.

mouth and jaw, and thin lips. One patient [1] had a small penis and a congenital hydrocele. In this boy, closure of fontanelles and sutures was delayed; by 19 months they were all widely patent. A third fontanelle may be present. Another of our patients had even more delayed closure of sutures, but she was found to have cleidocranial dysplasia and apparently independent mutations in the RUNX2 gene (Figure 86.8).

Malabsorption was documented by an increase in quantified stool fat in two patients studied [6]. Biopsies of the duodenum revealed no abnormalities. Bile acids were found to be in normal concentration in serum, urine, and feces [6] and studies of the pool size, fractional turnover rates, and rates of synthesis of bile acids appeared to be normal [19].

Electroencephalograms were carried out in eight patients, five of which were normal, and three showed generalized slowing. Neuroimaging was most striking for the presence of cerebellar atrophy [6, 7]. In one patient, a normal magnetic resonance image (MRI) at 18 months when he was presymptomatic was followed over the next three years by the parallel development of severe ataxia and cerebellar atrophy. In some patients there was also some cortical atrophy. Neuropathologic examination in one patient confirmed loss of cerebellar mass, including the entire vermis. Histology of the muscle was consistent with atrophy. Myopathy or hypotonia may lead to kyphoscoliosis.

The recurrent crises of fever have been associated with vomiting and diarrhea. Careful search has failed to reveal infectious agents. They occurred up to 25 times per year

and averaged 4–5 days in duration. Four patients died during crises. Some have had arthralgia, subcutaneous edema and a cutaneous eruption during the crisis.

Most patients with HIDS exhibit only the recurrent febrile crises and do not have neurologic abnormalities or dysmorphic features [20]. On the other hand, elevated levels of IgD have been found in 100 percent of patients with the classic mevalonic aciduria phenotype [7], and they rise during acute febrile crises [13]; so do the acute phase reactants. Elevated IgD may be absent in a patient with neurologic features and retinal dystrophy and an absence of periodic fevers [7].

Anemia may be sufficiently severe that a number of blood transfusions are required [8]. Some degree of anemia was present in five of the nine patients on whom this information was available. The serum cholesterol concentration may be normal or slightly reduced. Abnormal levels were reported in four patients, but two of these also had normal levels on occasion [6]. The creatinine kinase levels in plasma were markedly elevated in a majority of patients. Values as high as 3000 and 7520 IU/L have been recorded. The highest levels were in the course of acute crises, but the peak elevation followed the peak of symptoms by 2–4 days. The level of CK was positively correlated with the urinary excretion of mevalonic acid. Transaminases (AST and ALT) were also elevated in a majority of patients. Metabolic acidosis is not a feature of this disease.

GENETICS AND PATHOGENESIS

The disorder is autosomal recessive in nature. Two affected offspring were observed in four families, and the sex distribution has been equal [6]. Furthermore, activity of mevalonate kinase intermediate between patient and control was found in lymphocytes freshly isolated from both the father and mother of patients [1, 2]. Prenatal diagnosis of an affected fetus was carried out in a subsequent pregnancy of the index patient by analyzing the amniotic fluid for mevalonic acid. The pregnancy was terminated and the diagnosis confirmed by assay of the enzyme in fetal tissues.

The molecular defect in mevalonic aciduria is in the enzyme mevalonate kinase (Figure 86.1). In the usual assay, ^{14}C -labeled mevalonic acid and adenosine triphosphate (ATP) are converted to labeled mevalonate phosphate and pyrophosphate [1, 2, 21, 22]. Control fibroblast lysates displayed a mean activity of 1380 pmol/min/mg protein. Lysates of fibroblasts derived from the patient had activities of 0–4 percent of the control mean. In lymphoblasts, activity in the patients was 1–2 percent of the control mean. The enzyme is also active in freshly isolated lymphocytes and defective activity has been documented in lymphocytes of patients [1]. The level of residual activity has correlated poorly with clinical phenotype; the mildest phenotype reported had 0.4 percent of control activity in fibroblasts [7]. In nine heterozygotes, mean activity in lymphoblasts

was 47 percent and in seven, activity in fibroblasts was 67 percent of the control mean.

The localization of the gene was more finely defined to 12q24 and narrowed to a 9 cm region [15]. A missense mutation was identified in the index patient with mevalonic aciduria [3], an A to C change at nucleotide 902 causing a change of asparagine 301 to threonine (N301T). He was a compound of this mutation and another considered to have been inherited from his mother. Two patients in another family with a relatively mild phenotype were homozygous for a G to A change at 1000 yielding a change from alanine 334 to threonine [4, 7]. Another patient was homozygous for I268T [23]. The patient with the mildest phenotype [7] was a compound of A334T and 72insT [7]. In patients with HIDS, four missense mutations and a 92 bp deletion were identified [14, 15]. One mutation (V337I) was found in nearly all of the patients studied, usually compound. Enzyme activity was reduced in all, but not completely deficient. Four novel mutations were found [24] in a cluster: L243I, L264F, L265P, and L268T, the last found in a Mennonite family. Bacterial expression assay confirmed the enzyme-deficient nature of these mutations.

Overall, a majority of mutations have been missense [5]. The mutation r.V377I continues to be relatively frequently encountered and exclusively associated with the HIDS (hyperimmunoglobulin D periodic fever syndrome), usually as one of two disease genes. Under *in vitro* conditions designed to improve protein folding in cultured fibroblasts, the phenotype of residual mevalonate kinase activity correlated well with that of enzyme protein indicated by immune blotting. Mutations p.V377I, p. A148T, p. N205D, and p.T 209A, along with temperature sensitivity, are considered primarily folding or stability of the enzyme. Patients with the classic mevalonic kinase phenotype tend to have very low residual activity in this assay, but even some of these exhibited culture conditions dependent increase in activity. The p.A334T mutation does appear to be a Km mutant, affecting the binding of substrate [5].

In the presence of the metabolic block, mevalonic acid accumulates in body fluids. Quantitative analysis of the urine of ten patients revealed a massive excretion ranging from 900 to 56,000 mmol/mol creatinine, while normal subjects excrete a mean of 0.16 mmol/mol creatinine. Excretions of 900–1700 mmol/mol creatinine were found in patients with milder disease. Plasma concentrations in patients have ranged from 30 to 540 mmol/L; the control mean was 0.026 mmol/L. Mevalonic acid clearance by the kidney is very efficient and it appears to involve active renal tubular secretion. A patient with a low level of mevalonic aciduria (51–69 mmol/mol creatinine) was found to have a relatively large amount of residual activity of the enzyme [21].

The pathogenesis of the clinical manifestations of mevalonic aciduria is not clear. Mevalonic acid occupies a unique place in intermediary metabolism. It is an important precursor in the biosynthesis of cholesterol and other sterols, dolichol, and ubiquinone, as well as nonsterol



Figure 86.8 EF: An infant with mevalonic aciduria. The forehead was long and a V-shaped open fontanel was visible. She was heterozygous for two mutations, p.P263R and p.I268T, performed by Dr HR Waterham of the University of Amsterdam, the first of which was novel. Mevalonate kinase activity was 0, performed by Dr Michael Gibson then of the University of Oregon. In addition she had cleidocranial dysplasia and a heterozygous mutation in the RUNX2 gene, p.Y394X, a novel mutation leading to a truncated protein reported by Dr Christine Eng at Baylor University.

isoprenes involved in the formation of membranes, the glycosylation of proteins, the respiratory chain, and the replication of DNA [25, 26] (Figure 86.2). The enzyme is present in peroxisomes as well as the cytosol.

The pathway is regulated via feedback inhibition by cholesterol of the synthesis of mevalonic acid at the 3-hydroxy-3-methylglutaryl-CoA (HMG CoA) reductase step. When cholesterol, which is ingested or derived from plasma low density lipoproteins (LDL), downregulates HMG CoA reductase, nonsterol isoprenoid synthesis is preserved by inhibition of squalene synthetase and more distal enzymes, further limiting the incorporation of farnesylpyrophosphate into cholesterol. The initial enzymes in the nonsterol branches of the pathway have a very high affinity for farnesylpyrophosphate [25, 27].

Provision of exogenous cholesterol was without evident effect on patients, indicating (along with their relatively normal levels of cholesterol) that the clinical disease is not a consequence of a shortage of cholesterol. On the other hand, a direct test of the hypothesis that mevalonic acid itself was toxic, by inhibition of HMG CoA reductase with lovastatin, resulted in a severe clinical crisis with rhabdomyolysis [6]. These observations have focused on the possibility of diminished synthesis of a nonsterol isoprenoid product of the pathway.

Ubiquinone concentrations in plasma were found to be reduced in four of six patients studied. Levels were consistently below the control range, though not very far below. Ubiquinone is important for cardiac and muscular function. Concentrations of leukotriene E4 in the urine

of patients were found to be highly elevated [6, 28]. Furthermore, in the two patients given lovastatin there was a further 20 percent reduction in ubiquinone.

The recognition that this defect causes HIDS could provide clues as to the pathogenesis of these diseases. Studies of 2^{14}C -mevalonate in rats indicated a difference in metabolism in brain and skin where there was formation of labeled fatty acids palmitate and stearate, through a postulated shunt mechanism, from liver where there was no labeling of fatty acids [29]. It has become clear that the elevated levels of leukotrienes and IgD are secondary [7]. In addition, the febrile crises appear to diminish with increasing age.

The observation that mutant mevalonic kinase activity can be increased in cultured fibroblasts by a chemical chaperone approach to improving protein folding [5] raises the possibility of therapeutic approaches to improving activity with small molecules.

TREATMENT

Trials of supplemental cholesterol, bile acids, and inhibitors of HMG CoA reductase have not been therapeutic. Corticosteroid therapy appears to ameliorate acute crises [6] and a trial of long-term intermittent steroid treatment is in progress. Supplementation with ubiquinone may be of interest.

Some success has been reported in treating febrile episodes with anakinra, the interleukin-1-receptor antagonist [30]. Allogeneic bone marrow transplantation has been reported [31] in a three-year-old who had sustained remission from febrile attacks and inflammation.

REFERENCES

1. Hoffmann G, Gibson KM, Brandt IK *et al*. Mevalonic aciduria. An inborn error of cholesterol and non-sterol isoprene biosynthesis. *N Engl J Med* 1986; **314**: 1610.
2. Hoffmann GF, Sweetman L, Bremer HJ *et al*. Facts and artefacts in mevalonic aciduria: development of a stable isotope dilution GCMS assay for mevalonic acid and its application to physiologic fluids, tissue samples, prenatal diagnosis and carrier detection. *Clin Chim Acta* 1991; **198**: 209.
3. Schafer BL, Bishop RW, Kratunis VJ *et al*. Molecular cloning of human mevalonic kinase and identification of a missense mutation in the genetic disease mevalonic aciduria. *J Biol Chem* 1992; **267**: 13 229.
4. Hinson DD, Chambliss KL, Hoffmann GF *et al*. Identification of an active site alanine in mevalonate kinase through characterization of a novel mutation in mevalonate kinase deficiency. *J Biol Chem* 1997; **272**: 26 756.
5. Saskia HL, Schneiders MS, Koster J, Waterham HR. Mutational spectrum and genotype-phenotype correlations in mevalonate kinase deficiency. *Hum Mutat* 2006; **27**: 796.

6. Hoffmann GF, Charpentier C, Mayatepek E *et al.* Clinical and biochemical phenotype in 11 patients with mevalonic aciduria. *Pediatrics* 1993; **91**: 915.
7. Prietsch V, Mayatepek E, Krastel H *et al.* Mevalonate kinase deficiency: Enlarging the clinical and biochemical spectrum. *Pediatrics* 2003; **111**: 258.
8. de Klerk JBC, Duran M, Dorland L *et al.* A patient with mevalonic aciduria presenting with hepatosplenomegaly, congenital anaemia, thrombocytopenia and leukocytosis. *J Inherit Metab Dis* 1988; **2**: 233.
9. Kozich V, Gibson KM, Zeman J *et al.* Mevalonic aciduria. *J Inherit Metab Dis* 1991; **14**: 265.
10. Mancini J, Philip N, Chabrol B *et al.* Mevalonic aciduria in 3 siblings: A new recognizable metabolic encephalopathy. *Pediatr Neurol* 1993; **9**: 243.
11. Cenedella RJ, Sexton PS. Probing cataractogenesis associated with mevalonic aciduria. *Curr Eye Res* 1998; **17**: 153.
12. Di Rocco M, Caruso U, Waterham HR *et al.* Mevalonate kinase deficiency in a child with periodic fever and without hyperimmunoglobulinaemia D. *J Inherit Metab Dis* 2001; **24**: 411.
13. Tsimaratos M, Kone-Paut I, Divry P *et al.* Mevalonic aciduria and hyper-IgD syndrome: two sides of the same coin? *J Inherit Metab Dis* 2001; **24**: 413.
14. Houten SM, Kuis W, Duran M *et al.* Mutations in MVK encoding mevalonate kinase cause hyperimmunoglobulinaemia D and periodic fever syndrome. *Nat Genet* 1999; **22**: 175.
15. Drenth JP, Cuisset L, Grateau G *et al.* Mutations in the gene encoding mevalonate kinase cause hyper-IgD and periodic fever syndrome. International Hyper-IgD Study Group. *Nat Genet* 1999; **22**: 178.
16. Divry P, Rolland MO, Zabot MT *et al.* Mevalonic kinase deficiency in 2 siblings. *Society for the Study of Inborn Errors of Metabolism*. Proceedings of the 29th Annual Symposium, London, Sept 1991: **146** (Abstr.).
17. Berger R, Smit GPA, Schierbeek H *et al.* Mevalonic aciduria: an inborn error of cholesterol biosynthesis? *Clin Chim Acta* 1985; **152**: 219.
18. Gibson KM, Hoffmann G, Nyhan W *et al.* Mevalonate kinase deficiency in a child with cerebellar ataxia, hypotonia and mevalonic aciduria. *Eur J Pediatr* 1988; **148**: 250.
19. Gibson KM, Stellaard F, Hoffmann GF *et al.* Bile acid metabolism in three patients with mevalonic aciduria due to mevalonate kinase deficiency. *Clin Chim Acta* 1993; **217**: 217.
20. Drenth JP, Haagsma CJ, van der Meer JW. Hyperimmunoglobulinemia D and periodic fever syndrome. The clinical spectrum in a series of 50 patients. International Hyper-IgD Study Group. *Medicine (Baltimore)* 1994; **73**: 133.
21. Gibson KM, Hoffmann GF, Sweetman L, Buckingham B. Mevalonate kinase deficiency in a dizygotic twin with mild mevalonic aciduria. *J Inherit Metab Dis* 1997; **20**: 391.
22. Hoffmann GF, Brendel SU, Scharfschwerdt SR *et al.* Mevalonate kinase assay using DEAE-cellulose column chromatography for first-trimester prenatal diagnosis and complementation analysis in mevalonic aciduria. *J Inherit Metab Dis* 1992; **15**: 738.
23. Houten SM, Wanders RJ, Waterham HR. Biochemical and genetic aspects of mevalonate kinase and its deficiency. *Biochim Biophys Acta* 2000; **1529**: 19.
24. Hinson DD, Ross RM, Krisans S *et al.* Identification of a mutation cluster in mevalonate kinase deficiency including a new mutation in a patient of Mennonite ancestry. *Am J Hum Genet* 1999; **65**: 327.
25. Brown MS, Goldstein JL. Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *J Lipid Res* 1980; **21**: 505.
26. Surani MA, Kimber SJ, Osborn JC. Mevalonate reverses the developmental arrest of preimplantation mouse embryos by Compactin, an inhibitor of HMB CoA reductase. *J Embryol Exp Morph* 1983; **75**: 205.
27. Faust JR, Goldstein JL, Brown MS. Synthesis of ubiquinone and cholesterol in human fibroblasts: regulation of a branched pathway. *Arch Biochem Biophys* 1979; **192**: 86.
28. Frenkel J, Willemsen MA, Weemaes CM *et al.* Increased urinary leukotriene E(4) during febrile attacks in the hyperimmunoglobulinaemia D and periodic fever syndrome. *Arch Dis Child* 2001; **85**: 158.
29. Edmond J, Popják G. Transfer of carbon atoms from mevalonate to n-fatty acids. *J Biol Chem* 1974; **249**: 66.
30. Takada K, Aksentjevich I, Mahadevan V *et al.* Favorable preliminary experience with etanercept in two patients with the hyperimmuno-globulinemia D and periodic fever syndrome. *Arth Rheum* 2003; **48**: 2645.
31. Neven B, Valayannopoulos V, Quartier P *et al.* Allogeneic bone marrow transplantation in mevalonic aciduria. *N Engl J Med* 2007; **356**: 26.

Lipoprotein lipase deficiency/type I hyperlipoproteinemia

Introduction	648	Treatment	653
Clinical abnormalities	648	References	654
Genetics and pathogenesis	651		

MAJOR PHENOTYPIC EXPRESSION

Creamy appearance of fasting plasma, episodic abdominal pain, pancreatitis, eruptive xanthomas, lipemia retinalis, hepatosplenomegaly, increased concentration of triglycerides in plasma, hyperchylomicronemia, type I hyperlipoproteinemia and deficient activity of lipoprotein lipase.

INTRODUCTION

Some of the clinical characteristics of familial hyperchylomicronemia were recognized as early as 1932 in the report by Burger and Grutz [1] of an 11-year-old boy, the product of a first-cousin mating, in whom cutaneous xanthomas and hepatosplenomegaly were associated with creamy fasting plasma. Holt and colleagues [2] in 1939 reported two siblings with gross hyperlipemia. The proband had

severe attacks of abdominal pain. The defect in lipoprotein lipase in postheparin plasma was defined by Havel and Gordon [3] in 1960 in a study of three siblings with fasting hyperchylomicronemia. The initial patient of Holt *et al.* [2] was followed by Knittle and Ahrens [4] and shown by Frederickson and colleagues [5] to have defective activity of postheparin lipoprotein lipase (EC 3.1.1.3) activity. The enzyme requires a cofactor, apolipoprotein CII, and catalyzes the hydrolysis of the triglycerides of chylomicrons. Clearance of chylomicrons from plasma is impaired leading to accumulation of triglycerides. Deficiency of the apoenzyme or the cofactor can cause clinical lipoprotein lipase deficiency. The gene (LPL) for lipoprotein lipase has been mapped to chromosome 8p22 [6], and it contains 10 exons over 30 kb [7]. Over 100 different mutations have been identified, the majority of them point mutations [8].

CLINICAL ABNORMALITIES

The earliest recognition of this disorder is often fortuitous. The lactescence of blood, plasma, or serum (Figure 87.1) is observed in a routine sample drawn for some other



Figure 87.1 Whole blood of an untreated patient with lipoprotein lipase deficiency. The sample appeared virtually uniformly creamy. With standing for 18 hours or more at 4°C, plasma of these patients displays a layer of cream above and a clear layer below.



Figure 87.2 JL: A 21-month-old infant with type I hyperlipoproteinemia. The face at the time of admission revealed a roughened patch on the cheek containing tiny, pearly xanthomata. These disappeared promptly after dietary lowering of triglyceride concentrations. The patient was admitted because of abdomen distension and found to have a hepatic mass, which was removed and was a benign hemangioendothelioma.



Figure 87.3 JL: Two xanthomata that appeared on the leg after poor compliance with diet.



Figure 87.4 A group of xanthomata on the skin of the same patient.



Figure 87.5 Small but plentiful xanthomata characterize lipoprotein lipase deficiency in this four-year-old patient with advanced cutaneous disease. Triglycerides ranged from 2000 to 4000 mg/dL.

purpose. In fact, the obtaining of blood samples is so common in developed countries that it is surprising most patients are not recognized in this way. The disorder may be evident as early as 2 days (Figures 87.2, 87.3, 87.4, and 87.5), 8 days [9], and one month [10].

At the other extreme, asymptomatic patients have been discovered in the course of screening family members. The blood looks like cream of tomato soup. The characteristic appearance of an excess of chylomicrons in the blood is demonstrated by permitting the plasma to stand for 18–24 hours at 4°C (Table 87.1). The chylomicrons appear as a creamy layer on top and the infranatant layer is clear. In types III, IV, and V hyperlipoproteinemia both layers are turbid, while in type II the plasma is clear throughout. A supernatant creamy layer is also seen in type V. The convention for these observations and for quantitative studies of lipid concentrations is that blood samples are obtained after a 12–14-hour fast. In addition, the individual should not have gained or lost weight unusually for 2 weeks previously, received an unusual diet, or taken any drugs known to affect lipid concentrations. The period of fasting is usually modified in young infants, although our infant withstood a 24-hour fast without hypoglycemia or other complication.

The most common clinical presentation is with acute, recurrent episodes of abdominal pain [11–13]. The age at which this symptomatology begins is quite variable, but it ultimately occurs in virtually all individuals with this disorder. It may occur first in infancy, somewhat later in mid-childhood [12], or not until 20 [10] or 25 years of age. Pains may vary from mild apparently infantile colic to severe peritonitis. They may be quite disabling. They are often generalized or mid-epigastric, but may be localized, especially to the right or left upper quadrant. Narcotic dependence has been observed. Pain may be associated with spasm, rigidity, or rebound tenderness. There may also be fever and leukocytosis, and this presentation has led to surgical intervention. Usually, a milky exudate in

Table 87.1 Lipoprotein patterns characteristic of inherited hyperlipidemias

Type	Current designation	Lipoprotein patterns				Appearance of plasma ^a	Lipid concentrations		
		Chylomicrons	VLDL	LDL	HDL		Cholesterol	Triglycerides	Chol/Tri ratio
I	Hyperchylomicronemia	Increase	N or ↑	N or ↓	N or ↓	Creamy on top clear below	N or Sl. ↑	↑	<0.2
II	Familial hypercholesterolemia	Absent	N(IIa) ↑(IIb)	↑	N	Clear, or Sl. turbid	↑	N	>1.5
III	'Broad or floating beta' disease	Absent	β-VLDL ^b	Abn. ^b	N	Turbid, or faint layer of cream	↑	↑	Variable May = 1
IV	Familial hyper-pre-β-lipoproteinemia	Absent	↑	N	N	Turbid, no layer of cream	↑ or N		Variable
V	Familial hyper-pre-β-lipoproteinemia and hyperchylomicronemia	Increased	↑	N	N	Creamy on top turbid below	↑		0.15–0.6

^aAfter standing for 18–48 hours at 4°C.

^b'Floating' β-VLDL: LDL of abnormal lipid composition.

Abn, abnormal; HDL, high density lipoprotein; LDL, low density lipoprotein; N, normal; Sl, slightly; VLDL, very low density lipoprotein.

the peritoneal cavity is the only finding. The viscera may appear pale or fatty. Pains may be accompanied by anorexia and abdominal distension, or by vomiting, or diarrhea.

Acute attacks are always associated with hyperlipemia. In a patient being successfully managed they may follow dietary indiscretion or noncompliance. They are especially likely to follow the resumption of a normal diet in an individual who had reduced triglyceride levels by dietary restriction. They may also follow intercurrent infection, and attacks have been related to alcohol. Pregnancy may severely exacerbate symptoms [10] and so may oral contraceptive agents. Many patients learn to regulate their dietary intake of fat in a manner sufficient to eliminate the occurrence of abdominal pains [12].

Acute pancreatitis is a well-recognized complication of hyperchylomicronemia [10, 14, 15]. This may cause severe abdominal pain radiating to the back or shoulders and prostration, hypotension, sweating, and shock. It may lead to complete pancreatic necrosis, but does not lead to calcification. Pancreatitis may be fatal [10]. Necrotizing pancreatitis may be recognized at surgery [14], and pancreatic pseudocysts may be found [10], as well as extensive mesenteric fat necrosis and multiple adhesions. The serum amylase level may be very high. On the other hand, the diagnosis of pancreatitis in these patients may be complicated by the fact that the turbid plasma of the patient with hyperchylomicronemia may interfere with the determination of amylase activity in the serum [16, 17]. Normal amylase values have been observed in patients documented at laparotomy to have pancreatitis [18].

These problems may be overcome by serial dilution of the serum if the lactescence is recognized and a true elevation demonstrated [16], or probably better by examination of amylase in the urine, especially the amylase/creatinine clearance ratio [17]. Pancreatitis has been clearly related to

the presence of hyperlipemia [2, 14], and dietary reduction of serum triglyceride levels is successful in preventing further attacks. In fact, attacks of pancreatitis appear to occur only when serum concentrations of triglycerides exceed 1000 mg/dL [15], and morbidity and mortality are rare when levels are under 2000 mg/dL [18]. The association between hyperchylomicronemia and pancreatitis is so close that patients with diagnosed pancreatitis or recurrent abdominal pain should be screened for hyperlipemia. In 45 patients with acute pancreatitis examined prospectively, ten were found to have hyperlipoproteinemia [15]. Certainly, infants and children with pancreatitis and patients with familial pancreatitis should be examined for hyperchylomicronemia. In a 31-year clinical follow up of a patient who had 22 episodes of recurrent pancreatitis, he had nevertheless preserved pancreatic endocrine function, no pseudocysts, and no pancreatic calcification [19].

Hepatosplenomegaly is common in type I hyperlipoproteinemia [20]. It may be particularly prominent in infants and children. It is clearly related to fat intake, and the size of these organs can decrease within 24–48 hours of the initiation of the fat-free diet. Generally, some enlargement remains even with long-term dietary management. Occasionally, pains have been related to the spleen, and the spleen may be quite hard. It also recedes in size with reduction in the intake of fat. Fat embolism may occur in hyperlipemic individuals, and a variety of complications such as seizures, transient paralysis, or gastrointestinal hemorrhage have been attributed to such aggregations of chylomicrons. In one patient, what appeared to be splenic infarcts were seen on angiography, but at surgery the patient had pancreatitis and the removed spleen contained foam cells, but no infarcts [21]. Foam cells have been observed on needle biopsy of the liver [14], representing storage of lipid in macrophages and Kupffer cells.

Among early manifestations in 14 infants with the onset of symptoms prior to one year of age were irritability in seven, lower intestinal bleeding in two, splenomegaly in one, pallor or anemia in four [22]. In this series one additional patient came to light because of a positive family history, and another was discovered fortuitously. The intestinal bleeding stopped with institution of a low fat diet. Each patient had lactescent plasma at presentation.

Cutaneous eruptive xanthomas (Figures 87.3, 87.4 and 87.5) have been observed in about 50 percent of patients with type I hyperlipoproteinemia [20]. They cluster preferentially over the buttocks, proximal portions of the extremities and extensor surfaces, but they may occur anywhere, including the skin of the face. Lesions have been seen on the mucous membranes, including the hard palate and tonsils or fauces. They appear as nodules 1–5 mm in diameter. They result from phagocytosis of chylomicrons by macrophages [23]. They may be yellow or have a yellow center, but they may not. They may be erythematous; they tend to be flat. They may coalesce to form larger plaques. However, patients with this disease do not develop tendinous, tuberous, or planar xanthomas, or xanthelasma. The lesions are usually neither painful nor pruritic. They may occur within days of the elevation of plasma triglyceride levels over 2000 mg/dL and have been described as early as the first weeks of life [13]. They may fade rapidly on dietary reduction of these levels, but complete disappearance may take as long as 3 weeks.

Lipemia retinalis (Figure 87.6) is seen occasionally, but is characteristic of long-standing hyperlipemia. The entire fundus may have a pale or salmon cast, and there may be an increased light reflex over the vessels. The arteries and veins may appear milky-white. There may rarely be white deposits of lipid in the retina; and disturbances of circulation such as microaneurysms and hemorrhages have been reported [24, 25].

It is of interest that patients with type I hyperlipoproteinemia do not appear to be at risk for premature

atherosclerotic disease. The numbers of autopsied patients have been small, but none have had appreciable atherosclerotic change at ages ranging from 24 to 42 years of age [10]. Certainly, there has been no clinical evidence of coronary artery disease or cerebral vascular disease [19].

Some patients have been anemic [22], and one patient has been reported with persistent thrombocytopenia, leukopenia, and occasional anemia [26]. Bruising has been reported in another [27]. Slight to moderate hemolysis may be relatively common [28]. One patient [2] had chronic leg ulcers. A group of five patients has been reported with an unusual problem of intermittent swelling of the scrotum, and swelling, along with blueness or mottling, of the legs [12]. Surgical exploration of the scrotum revealed a milky effusion in the tunica vaginalis.

These patients, unlike those with many forms of hypertriglyceridemia, do not have abnormal glucose tolerance curves, and they do not have hyperuricemia. Secondary diabetes or pancreatic exocrine insufficiency may develop after many attacks of pancreatitis.

The very high plasma lipid may produce artifactual lowering of the values of many plasma solutes, determined in the routine clinical chemistry laboratory. The degree of error is approximately 1 percent for each 0.9 g triglyceride/dL [29]. Thus, in a patient with triglyceride of 10,000 mg/dL, an 11 percent reduction would yield a sodium concentration value of 129 mEq/L for a true sodium concentration of 145 mEq/L in fat-free plasma water. The importance of recognizing this issue is that such patients should not be treated for hyponatremia. On the other hand, lipemia may spuriously elevate levels of hemoglobin and bilirubin [30].

GENETICS AND PATHOGENESIS

Lipoprotein lipase deficiency is autosomal recessive in inheritance. Occurrence in a number of siblings has been reported [2], as has consanguinity [9]. Lipoprotein lipase activity of about 50 percent of normal has been reported in adipose tissue of parents of patients with deficiency [31]. Low levels of a lipolytic activity have also been observed in postheparin plasma of relatives, but heterozygosity cannot always be demonstrated by assay of the plasma [13]. Heterozygotes may have hypertriglyceridemia [31], but fasting levels of triglycerides are usually normal. In fact, it has been demonstrated by careful study of an extended pedigree [13] that hypertriglyceridemia of many genetic and other causes is so common in adults that the finding of an elevated concentration of triglycerides in a parent or relative cannot be equated with heterozygosity for lipoprotein lipase deficiency.

Analysis of the lipids of the plasma in patients reveals markedly elevated concentrations of triglycerides. In the untreated patient, levels usually range from 1000 to 4000 mg/dL but may be as high as 15,000 mg/dL



Figure 87.6 Lipemia retinalis in a patient with lipoprotein lipase deficiency.

[29]. Triglycerides constitute 80–95 percent of chylomicrons. Concentrations of cholesterol are normal or moderately elevated. It is only when the triglycerides are very high that the cholesterol rises; the ratio of the cholesterol to triglyceride is always less than 0.2 in type I hyperlipoproteinemia, and often less than 0.1.

Lipoprotein electrophoresis yields a characteristic chylomicron band at the origin. The type I pattern can be demonstrated by electrophoresis or ultracentrifugation as consisting exclusively, or nearly so, of chylomicrons (Table 87.1). The very low density lipoproteins (VLDL) are normal or slightly increased and the LDL and HDL are usually depressed. Treatment with a low fat, high carbohydrate diet usually leads, as chylomicrons fall, to an increase toward normal of LDL and increased levels of VLDL, but those of HDL remain low. The diagnosis of type I hyperlipoproteinemia is often confirmed by the elimination of fat from the diet, after which the chylomicrons disappear from the blood within a few days and triglyceride concentrations fall to 200–400 mg/dL. Most pediatric patients with hyperchylomicronemia have type I hyperlipoproteinemia. Most patients with type V are adults. However, childhood type V hyperlipoproteinemia has been reported [32], and patients with classic lipoprotein lipase deficiency sometimes have a type V pattern with time. Incubation of plasma in 3 percent polyvinylpyrrolidone will separate chylomicrons from other lipoproteins and is thus useful for the diagnosis of hyperchylomicronemia.

The definitive diagnosis of the classic type I disease requires demonstration of the molecular defect in the activity of the enzyme lipoprotein lipase [33–35]. This enzyme catalyzes the hydrolysis of glycerolester bonds in circulating triglycerides at the vascular endothelial surface in tissues, especially adipose. The enzyme is released by the intravenous administration of 60–100 units/kg heparin and is assayed in plasma obtained 10–15 minutes later. The enzyme requires the specific plasma cofactor, apoprotein C-II. It is inhibited by protamine. Concentrations of enzyme in patients are usually less than 10 percent of control levels and may approximate zero. Heparin was originally observed [36, 37] to clear postprandial lipemia in dogs. *In vitro* addition to plasma does not reproduce this effect; injection *in vivo* releases the lipase.

Defective activity of the enzyme may be documented in postheparin plasma or in adipose tissue. Heparin also releases hepatic lipase into plasma. This enzyme has little activity against chylomicrons, and so it created no problem in the original assay of Havel and Gordon [3], but most modern assays are done with artificial emulsions of triglycerides. Selective assay requires inhibition of the lipoprotein lipase with protamine or concentrated saline and calculating the difference from total lipolytic activity [38], inhibition of the hepatic lipase by specific antiserum [31], or chromatographic separation of two enzymes [39]. In classic lipoprotein lipase deficiency, patients have marked triglyceridemia and virtually always have clinical

symptoms before puberty, and they have defective enzyme activity in every tissue studied [40]. The enzyme may be measured in adipose tissue obtained by needle aspiration, which is of advantage because it does not contain hepatic lipase, but the assay must be done immediately thereafter. In patients with classic deficiency the activity in adipose tissue is defective whether the patient is in the fed or fasted state [41].

Some individuals have been observed in whom there was a partial deficiency of the enzyme [40]. A form of familial dominantly inherited hyperchylomicronemia has been reported in which there was a circulating inhibitor of lipoprotein lipase activity [42]. Another patient has been described [43] in whom a transient deficiency of lipoprotein lipase led to an attack of acute pancreatitis.

The enzyme is a homodimeric glycoprotein with identical 60 kDa subunits [44]. In many patients with deficient enzyme activity an immunochemically detectable protein is present, but a few patients have had no lipase protein [45]. The first of these was in a patient with no enzyme activity or cross-reactive material who had a 6 kb deletion involving exons 3–5 on one allele and a 2 kb deletion in exon 6 on the other [46, 47]. No mRNA could be found in adipose tissue.

The cDNA for the human enzyme codes for a mature protein of 448 amino acids. There is alternate splicing in adipose tissue, which produces two mRNAs [48, 49]. The mRNA is found in muscle, kidney, adrenal, intestine, and neonatal liver, as well as adipose tissues, but it is not found in adult liver.

Since the patient with the 6 kb deletion, a number of mutations has been identified, a majority of them missense [8, 50–52], but coding for very reduced or absent enzyme activity. A point mutation in exon 5 was found to account for the majority of alleles in the French-Canadian population, where prevalence is the highest in the world [22, 53, 54]. A C-to-T transition at nucleotide 875 led to a change from proline 207 to leucine. Dot blot analysis is available and a restriction site is available for analysis. G188E was common in Europe. Missense mutations have been found in 28 instances. Stop-codons have been produced by five single-base changes. A 3 kb deletion in exon 9 has been reported [55] and two splice site defects have occurred in intron 2 [56, 57]. The majority of missense mutations have been in exon 5, and in exons 4 and 6, areas of considerable homology among lipases. Some mutations have converted hydrophobic residues to less hydrophobic amino acids. Among mutations found in Japanese, a novel complex deletion insertion mediated by repetitive Alu elements led to the elimination of exon 2 [8]. Direct sequencing of the coding region of the LPL gene has detected some 97 percent of those with LPL deficiency. Mutations are also detectable by deletion/duplication analysis.

Phenotypic expression in heterozygotes was reported [58] in a pedigree of a proband homozygous for a mutation, G to A at position 818 leading to a glycine 188 to glutamic

acid substitution which resulted in an immunoreactive but nonfunctional enzyme. Heterozygotes had increased plasma triglyceride, VLDL cholesterol, and apolipoprotein B and decreased LDL and HDL cholesterol, clearly distinguished from noncarriers only after 40 years of age.

Differential diagnosis – apolipoprotein

C-II DEFICIENCY

A distinct molecular abnormality in the lipoprotein lipase enzyme complex has been defined as a deficiency in the apolipoprotein C-II activator of the complex [59–65]. These patients tend to present clinically later than those with classic lipoprotein lipase deficiency, in post-adolescence or adult life. The nature of the defect was suggested in the first patient who had displayed hyperlipemia and no activity of lipoprotein lipase, when the concentration of triglycerides fell sharply following a transfusion of blood for anemia. It was demonstrated that his plasma completely lacked apoC-II.

Patients with this disorder have had abdominal pains and pancreatitis. None have been described with xanthomas or hepatomegaly [62]. A few have had splenomegaly, and about half have had anemia. The pattern of hyperchylomicronemia is the same as in classic lipoprotein lipase deficiency [61]. The deficiency has most often been documented by the assay of lipoprotein lipase in the presence and absence of apoC-II or normal plasma. It can be assayed directly by electrophoresis of the tetramethylurea-soluble apoC-II on polyacrylamide gel.

The two molecularly defined genetic disorders, apoC-II deficiency and lipoprotein lipase deficiency, are rare. The most common causes of milky plasma are acquired and they are secondary to such disorders as diabetes, nephrosis, or alcoholism. Some have been associated with systemic lupus erythematosus, malignant histiocytosis, or lymphoma. In addition, there are clearly familial examples of hypertriglyceridemia and patients with typical symptomatic type I hyperlipoproteinemia in whom no molecular defect can be defined. Of 123 patients studied for hypertriglyceridemia, 110 were acquired, and eight fell into this latter category [33]. Only five had an abnormality in lipoprotein lipase, and these studies were carried out in the laboratory most of us rely on for assays for lipoprotein lipase.

In apoC-II deficiency, biochemical data are consistent with pedigree information and an autosomal recessive mode of transmission [63]. The gene for apoC-II has been mapped to chromosome 19 [64] and contains four exons spanning 3.3 kb [66]. Patients to date have been homozygous for a single mutation and are often products of a consanguineous mating. The nature of mutation has been defined in many of the small number of kindreds so far described [51]. In four, single-base change has led to a stop-codon [66–70], and in one there was a single-

base substitution in the methionine initiation codon [70]. A donor splice mutation intron 2 [71] rounds out this group, none of whom had demonstrable apoC-II protein on immunoassay. Four frame shift mutations would be expected to lead to truncated proteins; two of them had no detectable protein [72–77]. In a patient with the homozygous C1118A change in the APOC2 gene, which resulted in a Y63X, the patient had lipid encephalopathy, fatty deposits on cranial MRI, neurological impairment, and impaired mental development [78].

TREATMENT

The dietary restriction of the intake of fat has a dramatic effect in clearing hyperchylomicronemia and the avoidance of all of the manifestations of the disease. The argument for treatment is the substantial morbidity and mortality from pancreatitis. This should be eliminated along with the abdominal pains if concentrations of triglycerides are kept below 2000 mg/dL [79]. Most recommendations [29] are to keep levels below 1000 or even 750 mg/dL. It is generally agreed that symptoms will not occur at these levels. Both saturated and unsaturated fats must be restricted. Overall restriction should be to less than 15 percent of the calories from fats. In adults, diets containing less than 50 g of fat a day are usually sufficient. A value of 0.5 g/kg is useful in initiating therapy in children. An exception to restriction of fats is medium-chain triglycerides, which do not contribute to chylomicrons [80]. Diets extremely low in fat are well tolerated and consistent with normal growth and development (Figure 87.7).



Figure 87.7 LS: A 12-year-old girl with lipoprotein lipase deficiency. She had had episodes of pancreatitis, but she was well despite a lifelong severe restriction of the intake of fat.

Triglyceride levels should be studied throughout the day, not simply after an overnight fast. The regimen should ensure compliance around the clock. No single meal should contain more than 20 g of fat. At the same time, deficiency of essential fatty acids must be avoided. The management of an infant or child with type I hyperlipoproteinemia can be very difficult. Triglyceride levels may rise suddenly from a few hundred to several thousand mg/dL following a single fat-filled meal [3]. Agents known to increase concentrations of triglycerides, include alcohol, estrogens, including oral contraceptives [22], diuretics, isotretinoin, Zolof, and β -adrenergic blockers. Extreme dietary fat restriction during pregnancy has resulted in normal offspring. In the patients followed for over 30 years [19] the threshold level of triglycerides to trigger pancreatitis appeared to reduce with time. Despite progressive reduction in the insulin response to a glucose load, plasma glucose levels and that of hemoglobin A1c were not diabetic. Antioxidant therapy has been reported [81] to reduce the frequency of pancreatitis. Experience with treatment with diets highly restricted in fat beginning at less than one year of age indicated no adverse effects on growth [22].

Management of the acute abdominal pain requires vigilance about the diagnosis of pancreatitis and recognition that amylase values may be normal. The treatment of pancreatitis should follow the usual conservative regimen, with the additional precept that fat should be eliminated. In a neonate with chylomicronemia and congestive cardiac failure, triglyceride levels of 38,000 mg/dL were reduced to normal by plasmapheresis, and cardiac function became normal [82]. The treatment of apoC-II deficiency should generally be the same as that of lipoprotein lipase deficiency. An episode of pancreatitis may be successfully treated in apoC-II deficiency by the infusion of normal human plasma.

REFERENCES

- Burger M, Grutz O. Über hepatosplenomegale lipoidose mit xanthomatösen veränderungen in haut und schleimhaut. *Arch Dermatol Syph* 1932; **166**: 542.
- Holt LE Jr, Aylward FX, Timbres HG. Idiopathic familial lipemia. *Bull Johns Hopkins Hosp* 1939; **64**: 279.
- Havel RJ, Gordon RS Jr. Idiopathic hyperlipemia: metabolic studies in an affected family. *J Clin Invest* 1960; **39**: 1777.
- Knittle JL, Ahrens EH Jr. Carbohydrate metabolism in two forms of hyperglyceridemia. *J Clin Invest* 1964; **43**: 485.
- Fredrickson DS, Ono K, David LL. Lipolytic activity of postheparin plasma in hyperglyceridemia. *J Lipid Res* 1963; **4**: 24.
- Sparks RS, Zollner S, Klisak I *et al*. Human genes involved in lipolysis of plasma lipoproteins: mapping of loci for lipoprotein lipase to 8p22 and hepatic lipase to 15q21. *Genomics* 1987; **1**: 138.
- Deeb SS, Peng R. Structure of the human lipoprotein lipase gene. *Biochemistry* 1989; **28**: 4131.
- Okubo, M, Horinishi, A, Saito, M *et al*. A novel complex deletion-insertion mutation mediated by Alu repetitive elements leads to lipoprotein lipase deficiency. *Mol Genet Metab* 2007; **92**: 229.
- Sadan N, Drucker MM, Arger I *et al*. Type I hyperlipoproteinemia in an 8-day-old infant. *J Pediatr* 1977; **90**: 775.
- De Gennes JL, Menage JJ, Truffert MJ. Hyperglyceridemie exogene (hyperchylomicronemie) essentielle de type I: etude clinique et évolutive de cinq observations. *Nour Presse Med* 1972; **1**: 1835.
- Bloomfield AL, Shenson B. The syndrome of idiopathic hyperlipemia with crises of violent abdominal pain. *Stanford Med Bull* 1947; **5**: 185.
- Deckelbaum RJ, Dupont C, LeTarte J, Pencharz P. Primary hypertriglyceridemia in childhood. *Am J Dis Child* 1983; **137**: 396.
- Wilson DA, Edwards CO, Chan IF. Phenotypic heterogeneity in the extended pedigree of a proband with lipoprotein lipase deficiency. *Metabolism* 1983; **32**: 1107.
- Klatskin G, Gordon M. Relationship between relapsing pancreatitis and essential hyperlipemia. *Am J Med* 1952; **12**: 3.
- Farmer RG, Winkelman EI, Brown HB, Lewis LA. Hyperlipoproteinemia and pancreatitis. *Am J Med* 1973; **54**: 161.
- Fallat RW, Vester JW, Glueck CJ. Suppression of amylase activity by hypertriglyceridemia. *JAMA* 1973; **224**: 1331.
- Lesser PB, Warshaw AL. Diagnosis of pancreatitis masked by hyperlipemia. *Ann Intern Med* 1975; **82**: 795.
- Brunzell JD, Schrott HG. The interaction of familial and secondary causes of hypertriglyceridemia: role in pancreatitis. *Trans Assoc Am Physicians* 1973; **86**: 245.
- Kawashiri MA, Higashikata T, Mizuno M *et al*. Long-term course of lipoprotein lipase (LPL) (Arita) in a patient with recurrent pancreatitis, retained glucose tolerance, and atherosclerosis. *J Clin Endocrinol Metab* 2005; **90**: 654.
- Lees RS, Wilson DE, Schonfeld G, Fleet S. The familial dyslipoproteinemias. In: Steinberg AG, Bearn AG (eds). *Progress in Medical Genetics*, vol 9. New York: Grune and Stratton, 1973: 237.
- Ferrans VJ, Roberts WC, Levy RI, Fredrickson DS. Chylomicrons and the formation of foam cells in type I hyperlipoproteinemia. A morphologic study. *Am J Pathol* 1973; **70**: 253.
- Feoli-Fonseca JC, Levy E, Godard M, Lambert M. Familial lipoprotein lipase deficiency in infancy: clinical biochemical and molecular study. *J Pediatr* 1998; **133**: 417.
- Parker F, Bagdade JD, Odland GF, Bierman EL. Evidence for the chylomicron origin of lipids accumulating in diabetic eruptive xanthomas: a correlative lipid biochemical histochemical and electron microscopic study. *J Clin Invest* 1970; **49**: 2172.
- Moreau PG, Pichon P, Rife G. Manifestations chorioretiniennes des hyperlipidemies. La retinopathie hyperlipidémique a propos de 44 observations. *Sem Hop* 1970; **46**: 3467.
- Henkens HE, Houtsmuller AJ, Bos PJM, Crone RA. Fundus changes in primary hyperlipaemia. *Ophthalmologica* 1976; **173**: 190.

26. Romics L. Hypertriglyceridemia associated with thrombocytopenia and lipoprotein lipase deficiency. *Ann Intern Med* 1981; **95**: 660.
27. Potter JM, MacDonald WB. Primary type I hyperlipoproteinaemia – a metabolic and family study. *Aust NZ J Med* 1979; **9**: 688.
28. Cantin B, Boudriau S, Bertrand M *et al*. Hemolysis in primary lipoprotein lipase deficiency. *Metabolism* 1995; **44**: 652.
29. Brown WV, Baginsky ML, Ehnholm C. Primary type I and type V hyperlipoproteinaemia. In: Rifkind BM, Levy RI (eds). *Hyperlipidemia: Diagnosis and Therapy*. New York: Grune and Stratton, 1977: 93.
30. Shah PC, Patel AR, Rao KR. Hyperlipemia and spuriously elevated hemoglobin levels. *Ann Intern Med* 1975; **82**: 382.
31. Harlan WR Jr, Winesett PS, Wasserman AJ. Tissue lipoprotein lipase in normal individuals and in individuals with exogenous hypertriglyceridemia and the relationship of this enzyme to assimilation of fat. *J Clin Invest* 1967; **46**: 239.
32. Kwiterovich PO, Farah JR, Brown WV *et al*. The clinical biochemical and familial presentation of type V hyperlipoproteinemia in childhood. *Pediatrics* 1977; **59**: 513.
33. Brunzell JD, Bierman EL. Chylomicronemia syndrome. Interaction of genetic and acquired hypertriglyceridemia. *Med Clin N Am* 1982; **66**: 455.
34. Nilsson-Ehle P. Measurements of lipoprotein lipase activity. In: Borensztajn J (ed.). *Lipoprotein Lipase*. Chicago: Evener Publishers, 1987: 59.
35. Iverius P-H, Ostlund-Lindqvist A-M. Preparation characterization and measurement of lipoprotein lipase. *Methods Enzymol* 1986; **129**: 691.
36. Hahn PF. Abolishment of alimentary lipemia following injection of heparin. *Science* 1943; **98**: 19.
37. Eckel RH. Lipoprotein lipase: A multifunctional enzyme relevant to common metabolic diseases. *N Engl J Med* 1989; **320**: 1060. Erratum: *N Engl J Med* 1990; **322**: 477.
38. Ehnholm C, Shaw W, Greten H, Brown WV. Purification from human plasma of a heparin-released lipase with activity against triglyceride and phospholipids. *J Biol Chem* 1975; **250**: 6756.
39. Boberg J, Augustin J, Baginsky ML *et al*. Quantitative determination of hepatic and lipoprotein lipase activities from human postheparin plasma. *J Lipid Res* 1977; **18**: 544.
40. Brunzell JD, Chait A, Nikkila EA *et al*. Heterogeneity of primary lipoprotein lipase deficiency. *Metabolism* 1980; **29**: 624.
41. Goldberg AP, Chait A, Brunzell JD. Postprandial adipose tissue lipoprotein lipase activity in primary hypertriglyceridemia. *Metabolism* 1980; **29**: 223.
42. Brunzell JD, Miller NE, Alaupovic P *et al*. Familial chylomicronemia due to a circulating inhibitor of lipoprotein lipase activity. *J Lipid Res* 1983; **24**: 12.
43. Goldberg IJ, Paterniti JR Jr, Franklin BH *et al*. Transient lipoprotein lipase deficiency with hyperchylomicronemia. *Am J Med Sci* 1983; **286**: 28.
44. Olivecrona T, Bengtsson G, Osborne JC Jr. Molecular properties of lipoprotein lipase: effects of limited trypsin digestion on molecular weight and secondary structure. *Eur J Biochem* 1982; **124**: 629.
45. Brunzell JD, Iverius P-H, Scheibel MS *et al*. Primary lipoprotein lipase deficiency. In: Angel A, Frohlich J (eds). *Lipoprotein Deficiency Syndromes*. New York: Plenum Press, 1986: 227.
46. Langlois S, Deeb S, Brunzell JD *et al*. A major insertion accounts for a significant proportion of mutations underlying human lipoprotein lipase deficiency. *Proc Natl Acad Sci USA* 1989; **86**: 948.
47. Devlin RH, Deeb SS, Brunzell JD, Hayden MR. Partial gene duplication involving exon-Alu interchange results in lipoprotein lipase deficiency. *Am J Hum Genet* 1990; **46**: 112.
48. Enerback S, Bjursell G. Genomic organization of the region encoding guinea pig lipoprotein lipase; evidence for exon fusion and unconventional splicing. *Gene* 1989; **84**: 391.
49. Kirchgesner TG, Chaut JC, Heinzman C *et al*. Organization of the human lipoprotein gene and evolution of the lipase family. *Proc Natl Acad Sci USA* 1989; **86**: 9647.
50. Stenson PD, Ball EV, Mort M *et al*. Human Gene Mutation Database (HGMD). *Hum Mutat* 2003; **21**: 577.
51. Gilbert B, Rouis M, Griglio S *et al*. Lipoprotein lipase (LPL) deficiency: a new patient homozygote for the preponderant mutation Gly188Glu in the human LPL gene and review of reported mutations: 75% are clustered in exons 5 and 6. *Ann Genet* 2001; **44**: 25.
52. Henderson H, Ma Y, Kastelein J *et al*. Identification of the molecular defects underlying chylomicronemia in the majority of 75 separate probands with LPL deficiency. *Clin Res* 1991; **39**: 336A (Abstr.).
53. Bernstein R, Bocian M, Bengtsson U, Wasmuth J. Clinical application of fluorescent *in situ* hybridization techniques. *Clin Res* 1991; **39**: 96A.
54. Ma Y, Henderson HE, Ven Murthy MR *et al*. A mutation in the human lipoprotein lipase gene as the most common cause of familial chylomicronemia in French Canadians. *N Engl J Med* 1991; **324**: 1761.
55. Benlian P, Loux N, De Gennes JL *et al*. A homozygous deletion of exon 9 in the lipoprotein lipase gene causes type I hyperlipoproteinemia. *Arteriosclerosis* 1991; **11**: 1465.
56. Gotoda T, Yamada N, Murase T *et al*. Occurrence of multiple aberrantly spliced mRNAs upon a donor splice site mutation that causes familial lipoprotein lipase deficiency. *J Biol Chem* 1991; **266**: 24757.
57. Hata A, Emi M, Luc G *et al*. Compound heterozygote for lipoprotein lipase deficiency: Ser²⁴⁴Thr and transition in 3 splice site of intron 2(AG²⁴⁴AA) in the lipoprotein lipase gene. *Am J Hum Genet* 1990; **47**: 721.
58. Wilson DE, Emi M, Iverius P-H *et al*. Phenotypic expression of heterozygous lipoprotein lipase deficiency in the extended pedigree of a proband homozygous for a missense mutation. *J Clin Invest* 1990; **86**: 735.
59. Breckenridge WC, Little JA, Steiner G *et al*. Hypertriglyceridemia associated with deficiency of apolipoprotein C-II. *N Engl J Med* 1978; **298**: 1265.
60. Stalenhoef AFH, Casparie AF, Demacker PNM *et al*. Combined deficiency of apolipoprotein C-II and lipoprotein lipase in familial hyperchylomicronemia. *Metabolism* 1981; **30**: 919.
61. Fellin R, Baggion G, Poli A *et al*. Familial lipoprotein lipase and apolipoprotein C-II deficiency. Lipoprotein and apoprotein

- analysis, adipose tissue and hepatic lipoprotein lipase levels in seven patients and their first degree relatives. *Atherosclerosis* 1983; **49**: 55.
62. Little JA, Cox D, Breckenridge WC, McGuire VM. Introduction to deficiencies of apolipoproteins CII and EIII with some associated clinical findings. In: Gotto Jr AM, Smith LC, Allen B (eds). *Atherosclerosis V*. New York: Springer-Verlag, 1980: 671.
 63. Miller NE, Rao SN, Alaupovic P *et al*. Familial apolipoprotein C II deficiency: plasma lipoproteins and apolipoproteins in heterozygous and homozygous subjects and the effects of plasma infusion. *Eur J Clin Invest* 1981; **11**: 69.
 64. Humphries SE, Berg K, Gill L *et al*. The gene for apolipoprotein C-II is closely linked to the gene for apolipoprotein E on chromosome 19. *Clin Genet* 1984; **26**: 389.
 65. Fellin R, Baggio G, Poli A *et al*. Familial lipoprotein lipase and apolipoprotein C-II deficiency: lipoprotein and apoprotein analysis, adipose tissue and hepatic lipoprotein lipase levels in seven patients and their first degree relatives. *Atherosclerosis* 1983; **49**: 55.
 66. Wei C-F, Tsao Y-K, Robberson DL *et al*. The structure of the human apolipoprotein C-II gene: electron microscopic analysis of RNA:DNA hybrids complete nucleotide sequence and identification of 59 homologous sequences among lipoprotein genes. *J Biol Chem* 1985; **260**: 15 211.
 67. Baggio G, Manzato E, Gabelli C *et al*. Apolipoprotein C-II deficiency syndrome: clinical features lipoprotein characterization lipase activity and correction of hypertriglyceridemia after apo lipoprotein C-II administration in two affected patients. *J Clin Invest* 1986; **77**: 520.
 68. Crepaldi G, Fellin R, Baggio G *et al*. Lipoprotein and apoprotein adipose tissue and hepatic lipoprotein lipase levels in patients with familial chylomicronemia, and their immediate family members. In: Gotto Jr AM, Smith LC, Allen B (eds). *Atherosclerosis V*. New York: Springer-Verlag, 1980: 250.
 69. Fojo SS, De Gennes JL, Chapman J *et al*. A nonsense mutation in the apolipoprotein C-II_{Padova} gene in a patient with apolipoprotein C-II deficiency. *J Clin Invest* 1989; **84**: 1215.
 70. Fojo SS, De Gennes JL, Chapman J *et al*. An initiation codon mutation in the apo C-II gene (apo C-II_{Paris}) of a patient with a deficiency of apolipoprotein C-II. *J Biol Chem* 1989; **264**: 20 839.
 71. Fojo SS, Beisiegel U, Beil U *et al*. Donor splice site mutation in the apolipoprotein (apo) C-II gene (apo C-II_{Hamburg}) of a patient with apo C-II deficiency. *J Clin Invest* 1988; **82**: 1489.
 72. Fojo SS, Stalenhoef AF, Marr K *et al*. A deletion mutation of the apo C-II gene (apo C-II_{Nijmegen}) of a patient with a deficiency of apolipoprotein C-II. *J Biol Chem* 1988; **263**: 17 913.
 73. Yamamura T, Sudo H, Ishikawa K, Yamaoto A. Familial type I hyperlipoproteinemia caused by apolipoprotein C-II deficiency. *Atherosclerosis* 1979; **34**: 53.
 74. Matsuoka N, Shirai K, Johnson JD *et al*. Effects of apolipoprotein C-II (apo C-II) on the lipolysis of very low density lipoproteins from apo C-II deficient patients. *Metabolism* 1981; **30**: 818.
 75. Connelly PW, Maguire GF, Hofmann T, Little JA. Structure of apolipoprotein C-II Toronto, a nonfunctional human apolipoprotein. *Proc Natl Acad Sci USA* 1987; **84**: 270.
 76. Connelly PW, Maguire CF, Little JA. Apolipoprotein CII_{St Michael} familial apolipoprotein CII deficiency associated with premature vascular disease. *J Clin Invest* 1987; **80**: 1597.
 77. Xiong WJ, Li W-H, Posner I *et al*. No severe bottleneck during human evolution: evidence from two apolipoprotein C-II deficiency alleles. *Am J Hum Genet* 1991; **48**: 383.
 78. Wilson CJ, Oliva CP, Maggi F *et al*. Apolipoprotein C-II deficiency presenting as a lipid encephalopathy in infancy. *Ann Neurol* 2003; **53**: 807.
 79. Brunzell JD, Schrott HG. The interaction of familial and secondary causes of hypertriglyceridemia: Role in pancreatitis. *Trans Assoc Am Physicians* 1973; **86**: 245.
 80. Furman RH, Howard RP, Brusco OJ, Alaupovic P. Effects of medium chain length triglyceride (MCT) on serum lipids and lipoproteins in familial hyperchylomicronemia (dietary fat induced lipemia) and dietary carbohydrate-accentuated lipemia. *J Lab Clin Med* 1965; **66**: 912.
 81. Heaney AP, Sharer N, Rameh B *et al*. Prevention of recurrent pancreatitis in familial lipoprotein lipase deficiency with high-dose antioxidant therapy. *J Clin Endocrinol Metab* 1999; **84**: 1203.
 82. Bartuli A, Landolfo A, Pirozzi N *et al*. Severe chylomicronemia syndrome (CHS) with neonatal onset successful treatment of cardiac failure with plasmapheresis. *J Inherit Metab Dis* 2002; **25**(Suppl. 1): 161.

LIPID STORAGE DISORDERS

88.	Fabry disease	659
89.	GM ₁ gangliosidosis/ β -galactosidase deficiency	666
90.	Tay-Sachs disease/hexosaminidase A deficiency	678
91.	Sandhoff disease/GM ₂ gangliosidosis/deficiency of hexosaminidase A and B/hex-B subunit deficiency	686
92.	GM ₂ activator deficiency/GM ₂ gangliosidosis – deficiency of the activator protein	694
93.	Gaucher disease	698
94.	Niemann-Pick disease	708
95.	Niemann-Pick type C disease/cholesterol-processing abnormality	718
96.	Krabbe disease/galactosylceramide lipidosis/globoid cell leukodystrophy	726
97.	Wolman disease/cholesteryl ester storage disease	733
98.	Fucosidosis	740
99.	α -Mannosidosis	745
100.	Galactosialidosis	752
101.	Metachromatic leukodystrophy	760
102.	Multiple sulfatase deficiency	769

Fabry disease

Introduction	659	Treatment	662
Clinical abnormalities	659	References	663
Genetics and pathogenesis	661		

MAJOR PHENOTYPIC EXPRESSION

Angiokeratomas of the skin, episodic pain in the extremities, hypohidrosis, corneal and lenticular opacities, postprandial pain and diarrhea, neuropathy, renal disease, coronary and cerebral vascular disease, accumulation of glycosphingolipids with a terminal galactose, and deficiency of ceramide trihexosidase (α -galactosidase A).

INTRODUCTION

Fabry disease was first described in 1898 independently by Anderson [1] in England and Fabry [2] in Germany. The latter name has become its designation [3], possibly because Fabry continued to publish information about his patient over a 32-year period [4]. Anderson and Fabry each recognized the systemic nature of the disease but, as dermatologists, their focus was on the cutaneous angiokeratomas by which the patient is so readily recognized. The disease is also known as angiokeratoma corporis diffusum universale [5–7]. It was first noted to be X-linked by Opitz and colleagues in 1965 [7]. The disorder was appropriately classified as a glycosphingolipidosis following the isolation and characterization by Sweeley and Klionsky [8] of the

Fabry lipid as galactosylgalactosylglucosylceramide (Figure 88.1). The molecular defect was demonstrated by Brady and colleagues [9] as an inability to cleave the terminal galactose from this ceramide trihexoside; thus the defective activity is ceramide trihexosidase (Figure 88.1). The defective enzyme was shown by Kint [10] by means of an artificial substrate to be an α -galactosidase. It is referred to as α -galactosidase A to distinguish it from the α -N-acetylgalactosaminidase which is deficient in Schindler disease and is also an α -galactosidase (B). The gene is on the X chromosome at Xq22.1 [11]; the gene has been cloned and its sequence determined [12–14]. A large number and variety of mutations have been defined.

CLINICAL ABNORMALITIES

The initial symptom is usually pain occurring often within the first ten years of life (Table 88.1). It may be

Table 88.1 Clinical signs and symptoms of Fabry disease at different ages

Age	Signs
Childhood	Pain in extremities, fever, Fabry crisis
Adolescence	Angiokeratomas
Adulthood	Central nervous system symptoms Myocardial and pulmonary disease
Middle age	Renal failure, lymphedema

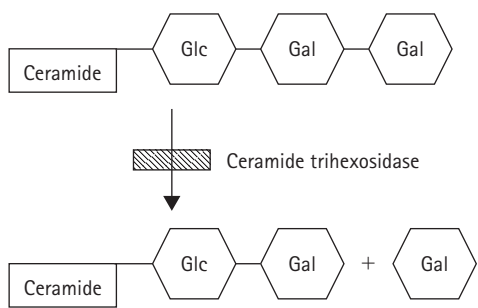


Figure 88.1 The enzyme ceramide trihexosidase is the site of the defect in Fabry disease. The reaction which cleaves a terminal galactose is a Q-galactosidase.

excruciating, has a burning quality, and tends to occur intermittently [15]. Pain is most often noted in the fingers and toes, or the hands and feet. There may be associated tingling acroparasthesias [16, 17]. An attack may be brief or may last for weeks. It may be induced by exposure to extreme heat or cold, fatigue or emotional stress. It may be associated with elevated body temperature and an elevated erythrocyte sedimentation rate, and patients have been diagnosed as having rheumatoid arthritis or rheumatic fever [18, 19]. Degeneration of the interphalangeal joints may lead to deformities. Abdominal or back pain may suggest appendicitis or renal colic [20]. Narcotics may not provide relief. Crises of pain are self-limited, disappearing spontaneously, only to return later. Pains may decrease in frequency and severity with age, and some older patients have no history of pain. Patients may also have recurrent episodes of fever.

It is the appearance of the skin lesions in adolescence or later that usually permits the diagnosis [5, 21]. These lesions are dark red punctate macules that do not blanch with pressure (Figures 88.2, 88.3, and 88.4). They occur



Figure 88.2 Angiokeratomas of the skin. These red-purple macules or maculopapules may feel hyperkeratotic. They are prominent on the hips, buttocks, and scrotum.



Figure 88.3 Angiokeratomas of the skin.



Figure 88.4 Angiokeratomas of the scrotum and penis of a 31-year-old patient.

in clusters and may be mistaken for petechiae. With time, some become papular and may feel rough to touch. There is some tendency for bilaterally symmetric distribution. The areas of most common involvement are the scrotum and buttocks, but they are also seen on the hips, back and thighs in a bathing-trunks distribution. The oral mucosa may also be involved. Microscopically the skin lesions are angiectatic lesions in the dermis with keratotic build-up superficially. These angiokeratomas are not usually symptomatic, but occasionally large lesions on the scrotum may bleed. The hands or feet or just the tips of the toes or fingers may be bright red, and sensitive to touch. Lymphedema may also be seen in the legs. Hypohidrosis



Figure 88.5 Tortuous, dilated, telangiectatic vessels of the conjunctiva. This man also had fine telangiectases of the facial skin.

or even absence of sweating is another dermatologic manifestation of the disease. Sweat pores may appear reduced. Patients are intolerant of heat and flush with exercise. One of our patients responded to hot weather by filling rubber boots with water and sloshing around in them, as well as soaking his head in cold water.

Ocular lesions [22,23] regularly include dilated tortuous venules of the conjunctivae ([Figure 88.5](#)). Similar dilatation may be seen in the vessel of the retinas. Corneal opacities develop in males and in some heterozygous females. The diagnosis can be made by slit lamp examination, in which the typical cream-colored interior, whorl-like opacities are visualized. Corneal opacities have been seen as early as six months of age [24]. Cataracts of the posterior capsule of the lens are pathognomonic [22]. The ocular lesions result from the deposition of glycosphingolipid and do not usually impair vision. As the disease progresses, the retinal changes of uremia may be found. Visual loss has been observed following central retinal artery occlusion [25]. Some patients display edema of the eyelids, in the absence of renal disease [22, 24]. Neurosensory hearing loss may develop [18].

Gastrointestinal manifestations may be prominent [26]. In some patients, they may be the only complaints for years. There may be postprandial pain or diarrhea. Infiltration of autonomic nerve cells and mucosal cells with lipid may interfere with peristalsis. Diverticula may develop, and rupture of a diverticulum is a surgical emergency.

The long-term complications of Fabry disease are consequences of the accumulation of glycosylsphingolipid in endothelial cells. The most regular concomitant of vascular dysfunction is chronic renal disease. The earliest manifestation is proteinuria, which usually occurs in the fourth decade. Hypertension is common. Examination of the urine may reveal red cells, casts and birefringent lipid globules forming maltese crosses within and outside cells, best seen under polarizing microscopy. Renal function gradually deteriorates, leading to renal failure. This usually occurs by the fifth decade, but may occur as early as 21. The concentration of creatinine in the blood increases linearly with time [18, 27]. Polyuria is a manifestation of defective concentrating ability [28]. Prior to the development of programs of hemodialysis, many hemizygotes died before 40 years of age [29].

Cardiac manifestations of vascular disease include myocardial ischemia or infarction. Cardiac symptoms include shortness of breath, angina or syncope resulting from arteriovenous (AV) block or ventricular outflow obstruction. Coronary occlusion or cerebral vascular disease often occurs before the age of 25 years [28, 30, 31]. Cardiac enlargement and myocardial failure may result from infiltration of the myocardium or the valves with lipid [30, 31]. Arrhythmias are also common. The PR interval is shortened [32]. Echocardiography may show increased thickness of the interventricular septum and the posterior wall of the left ventricle [33].

Cerebrovascular manifestations may be transient

ischemic attacks, strokes, seizures, hemiplegia, or aphasia. They result from infiltration and obstruction of cerebral vessels [34]. Magnetic resonance imaging (MRI) or proton magnetic resonance spectroscopy (MRS) imaging may reveal cerebrovascular disease [34, 35]. Some patients have had psychotic manifestations [36]. Abnormal cutaneous thermal sensation is common [34]. Elevated threshold for the detection of cold precedes that of warmth. Auditory and vestibular dysfunction increase with time [34].

Dyspnea on exertion is common, and airway obstruction may result from infiltration of bronchial epithelial cells [37]. Pulmonary function tests may show impairment. Other manifestations include lymphedema of the legs [38], priapism [39], and anemia [16]. Death usually results from uremia or from vascular disease of the heart or brain.

Heterozygous females have clinical manifestations with such frequency that the disease may be considered an X-linked dominant [40]. In a series of 20 heterozygotes none were asymptomatic [40, 41]. All but two had pain or burning either chronically or in Fabry crises. Ten had lymphedema and 11 angiokeratomata. Typical corneal lesions were seen in 14. MRI revealed multifocal infarcts or white matter disease. In a series of 60 obligate carriers, studied largely by questionnaire, serious or debilitating consequences were found in 30 percent and hypohidrosis in 33 percent, while pains occurred in 70 percent [41]. Cardiac involvement, especially left ventricular hypertrophy and valvular abnormalities were found to be common in a series of 35 female patients, and they progressed with age [42].

The pathology of Fabry disease consists of the widespread deposition of glycosphingolipid. Vacuoles are seen in a wide variety of cells, especially the endothelium of the blood vessels [43, 44]. Electronmicroscopy reveals a concentric or lamellar structure of the lysosomal inclusions.

GENETICS AND PATHOGENESIS

Fabry disease is an inborn error of glycosphingolipid metabolism transmitted in an X-linked character [7] which is far from fully recessive; the level of expression in the heterozygote ranges from asymptomatic to severity equal to that of the hemizygote.

The defective activity of α -galactosidase leads to the accumulation of glycosphingolipids that have a terminal α -galactosyl moiety [8, 45]. The most prominent is ceramide trihexoside ([Figure 88.1](#)), which is also known as globotriasylceramide [Gal(α 1 \rightarrow 4)Gal(β 1 \rightarrow 4)Glc(β 1-1')ceramide]. Galabiosylceramide, a compound in which ceramide is linked to two galactose moieties, is also found in large quantities in tissues such as kidney [23, 46]. The blood group B antigenic glycosphingolipid also contains a terminal galactose and, hence, it accumulates in Fabry patients with B or AB blood types [47].

The basic defect in Fabry disease is an inability to degrade

these glycosphingolipids because of defective activity of the enzyme that catalyzes the hydrolysis of the terminal galactose moieties [9]. The activity of α -galactosidase in most hemizygous males is less than 3 percent of normal, but as much as 20 percent of normal activity has been observed [48]. Studies with antibody against the enzyme have usually shown no cross-reactive material [49, 50]. α -Galactosidase is synthesized as a 50 kDa precursor protein, transported to the lysosome in a mannosphosphate receptor-dependent processing and cleaved to a mature 46 kDa enzyme [51]. Study of this sequence in cells of classic Fabry patients has indicated considerable variation: no enzyme precursor synthesized; precursor, but no mature proteins as a consequence of protein instability; and normal-appearing mature protein with no catalytic activity [51].

The gene for α -galactosidase has been localized to Xq22.1 [11]. X-linked inheritance was first established by pedigree analysis (Figure 88.6). Two populations of cells – one with normal galactosidase activity and the other defective – were shown by cloning of cultured fibroblasts [52]. The cDNA for the gene for α -galactosidase has been cloned and sequenced [12, 53], and this has permitted delineation of the nature of a number of mutations. The gene has seven exons. Major gene rearrangement detected by Southern hybridization included five deletions and a duplication [54, 55]. A number of smaller deletions and insertions have been identified, many of which led to frameshifts and premature termination. Most mutations in Fabry hemizygotes are not detected in this way. Mutations altering the processing of the mRNA transcript have been observed. Single nucleotide missense mutations have been identified in a majority of families [55–57], and most have been found only in a single family. However, a high frequency of mutation was observed at 14 CpG dinucleotides in the coding sequence. More than 300 mutations have been found in patients with Fabry disease [58]. Among them two novel mutations, 1277delAA(del2) and 1284delACTT(del4) in the 3' terminus, obliterated the termination codon and generated multiple transcripts, most of them inactive [58].

Fabry's disease
W. Kindred-La Jolla, 1970

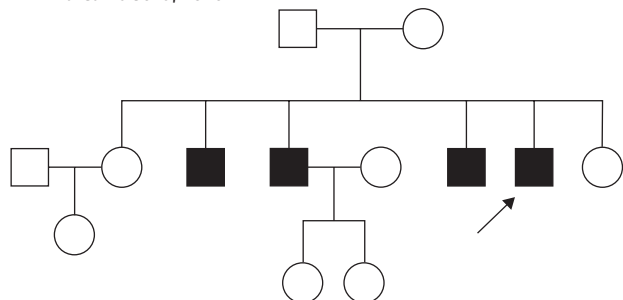


Figure 88.6 Pedigree of a family with four affected males. The pattern is of X-linked recessive inheritance.

Fabry disease is the most common lysosomal storage disease after Gaucher disease. The prevalence of heterozygous carriers in the United Kingdom was estimated at 1 in 339,000 females [41]. Heterozygote detection has been carried out by enzyme assay of cultured fibroblasts after cloning [52] and cell sorting, but these methods are not practical for clinical use. The assay of the enzyme in individual hair roots [59] is more convenient but still labor intensive. In a family in which the mutation has been identified, targeted analysis can be employed for precise identification of heterozygosity.

Prenatal diagnosis has been accomplished by the demonstration of deficient α -galactosidase activity in cultured amniotic fluid cells [60]. A microtechnique for α -galactosidase has been developed for prenatal diagnosis which requires small numbers of cultured amniocytes [61]. Diagnosis has also been made prenatally by chorionic villus sampling [62]. The identification of the molecular nature of mutation in a family permits this molecular technique to be used for prenatal diagnosis.

TREATMENT

Prevention and amelioration of the painful crises of the disease have been difficult, but diphenylhydantoin has been shown to be helpful; chronic low dosage has been employed (200–300 mg qd) [17]. Carbamazepine may also be helpful, and the combination of the two drugs may be particularly useful [63]. Gabapentin (Neurontin) has been recommended for this purpose [64]. Doses employed in adults have ranged from 100 mg bid to 300 mg bid, or 15–60 mg/kg in children. Neurotrophin, an extract of inflamed skin of vaccinia inoculated rabbits was reported to be as effective as carbamazepine in the usual leg pains; neither were effective in episodic colicky pain, but treatment with both eliminated it [65].

Chronic hemodialysis has been the mainstay of management of renal failure. Many patients have received kidney transplants following renal failure [65–68]. This solves the problem of renal failure, but does not alter the accumulation of lipid in other tissues. Some transplanted patients have survived long enough to die of cardiac disease. Enzyme replacement therapy with purified α -galactosidase [69] has been extended to trials with recombinant human enzyme, which have demonstrated safety and efficacy [70–72]. It is clear that treatment reverses the storage in lysosomes, which causes the disease. The product has been approved by the US Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal Products. It is marketed in the United States as Fabrazyme. Optimal dosage has yet to be determined. Doses employed have been 0.2 [71] to 1.0 [72] mg/kg (intravenously every 2 weeks for 20–22 weeks). Two products have been approved in Europe. They should have equal efficacy as neither mRNA is edited [73].

Activation of mutant enzymes by compounds such as

1-deoxygalactonojirimycin are being explored in novel approaches to the treatment of glycosphingolipidoses [74, 75]. This compound is an inhibitor of lysosomal α -galactosidase, but in low doses it serves as an activator increasing activity in mutant enzymes up to 14 times. These compounds have been referred to as chemical chaperones, because they accelerate transport and maturation of the enzyme molecule.

Another approach is substrate deprivation. Compounds such as D-erythro-1-ethylenedexyphenyl-2-palmitylamino-3-pyrrolidino-propanol (d-t-EtDO-P4) reduce accumulation of globotriosylceramide in tissues of murine Fabry models [76] by inhibiting the sphingolipid glucosyltransferase involved in their synthesis.

REFERENCES

- Anderson W. A case of angiokeratoma. *Br J Dermatol* 1898; **10**: 113.
- Fabry J. Ein Beitrag zur Kenntnis der Purpura haemorrhagica nodularis (Purpura papulosa hemorrhagica Hebrae). *Arch Dermatol Syph* 1898; **43**: 187.
- Aerts JMFG, Beck M, Cox TM. Fabry disease, new insights and future perspectives. *J Inherit Metab Dis* 2001; **24**(Suppl. 2): 1.
- Fabry J. Weiterer Beitrag zur Klinik des Angiokeratoma naeviforme (*Naevus angiokeratosus*). *Dermatol Schnschr* 1930; **90**: 339.
- Johnston AW, Weller SDV, Warland BJ. Angiokeratoma corporis diffusum. *Arch Dis Child* 1968; **43**: 73.
- Wallace RD, Cooper WJ. Angiokeratoma corporis diffusum universale (Fabry). *Am J Med* 1965; **39**: 656.
- Opitz JM, Stiles FCD, von Gemmingen G *et al*. The genetics of angiokeratoma corporis diffusum (Fabry's disease) and its linkage with Xg(a) locus. *Am J Hum Genet* 1965; **17**: 325.
- Sweeley CC, Klionsky B. Fabry's disease: classification as a sphingolipidosis and partial characterization of a novel glycolipid. *J Biol Chem* 1963; **238**: 3148.
- Brady RO, Gal AE, Bradley RM *et al*. Enzymatic defect in Fabry's disease – ceramidetrihexosidase deficiency. *N Engl J Med* 1967; **276**: 1163.
- Kint JA. Fabry's disease α -galactosidase deficiency. *Science* 1970; **167**: 1268.
- Shows TB, Brown JA, Haley LL *et al*. Assignment of alpha-galactosidase (alpha-GAL) gene to the q22-qter region of the X chromosome in man. *Cytogenet Cell Genet* 1978; **22**: 541.
- Bishop DF, Calhoun DH, Bernstein HS *et al*. Human α -galactosidase A: nucleotide sequence of a cDNA clone encoding the mature enzyme. *Proc Natl Acad Sci USA* 1986; **83**: 4859.
- Bishop DF, Kornreich R, Desnick RJ. Structural organization of the human alpha-galactosidase A gene: further evidence for the absence of a 3' untranslated region. *Proc Natl Acad Sci USA* 1988; **85**: 3903.
- Kornreich R, Desnick RJ, Bishop DF. Nucleotide sequence of the human α -galactosidase A gene. *Nucleic Acids Res* 1989; **17**: 3301.
- Wise D, Wallace HJ, Jellinek EH. Angiokeratoma corporis diffusum: a clinical study of eight affected families. *Q J Med* 1962; **31**: 177.
- Bagdale JD, Parker F, Ways PO *et al*. Fabry's disease: a correlative clinical morphologic and biochemical study. *Lab Invest* 1968; **18**: 681.
- Lockman LA, Hunnighake DB, Krivit W, Desnick RJ. Relief of pain of Fabry's disease by diphenyl-hydantoin. *Neurology* 1971; **23**: 871.
- Pyeritz RE, Bender WL, Lipford ED III. Anderson-Fabry disease. *Johns Hopkins Med J* 1982; **150**: 181.
- Sheth KJ, Bernhard GC. The arthropathy of Fabry disease. *Arth Rheum* 1979; **22**: 781.
- Rahman AN, Simcone FA, Hackel DB *et al*. Angiokeratoma corporis diffusum universale (hereditary dystopic lipidosis). *Trans Assoc Am Physicians* 1961; **74**: 366.
- Frost P, Spaeth GL, Tanaka Y. Fabry's disease – glycolipid lipidosis. Skin manifestations. *Arch Intern Med* 1966; **117**: 440.
- Sher NA, Letson RD, Desnick RJ. The ocular manifestations in Fabry's disease. *Arch Ophthalmol* 1979; **97**: 671.
- Brady RO. Ophthalmologic aspects of lipid storage diseases. *Ophthalmology* 1978; **85**: 1007.
- Spaeth GL, Frost P. Fabry's disease: its ocular manifestations. *Arch Ophthalmol* 1965; **74**: 760.
- Sher NA, Reiff W, Letson RD, Desnick RJ. Central retinal artery occlusion complicating Fabry's disease. *Arch Ophthalmol* 1978; **96**: 315.
- Rowe JW, Gilliam JI, Warthin TA. Intestinal manifestations of Fabry's disease. *Ann Intern Med* 1974; **81**: 628.
- Mitch WE, Walser M, Buffington GA, Lemann JR. A simple method of estimating progression of chronic renal failure. *Lancet* 1976; **2**: 1326.
- Parkinson JE, Sunshine A. Angiokeratoma corporis diffusum universale (Fabry) presenting as suspected myocardial infarction and pulmonary infarcts. *Am J Med* 1961; **31**: 951.
- Colombi A, Kostyal A, Bracher R *et al*. Angiokeratoma corporis diffusum-Fabry's disease. *Helv Med Acta* 1967; **34**: 67.
- Becker AR, Schoorl R, Balk AG, van der Heider RM. Cardiac manifestations of Fabry's disease. *Am J Cardiol* 1975; **36**: 829.
- Desnick RJ, Blieden L, Sharp HL *et al*. Cardiac valvular anomalies in Fabry disease. *Circulation* 1976; **54**: 818.
- Mehta J. Electrocardiographic and vectorcardiographic abnormalities in Fabry's disease. *Am Heart J* 1977; **93**: 699.
- Bass JL, Shrivastava S, Grabowski GA *et al*. The M-mode echocardiogram in Fabry's disease. *Am Heart J* 1980; **100**: 807.
- Morgan SH, Rudge P, Smith SJ *et al*. The neurological complications of Anderson-Fabry disease (alpha-galactosidase A deficiency) – investigation of symptomatic and presymptomatic patients. *Q J Med* 1990; **75**: 491.
- Moumdjian R, Tampieri D, Melanson D, Ethier R. Anderson-Fabry disease: a case report with MR, CT and cerebral angiography. *Am J Neuroradiol* 1989; **10**: S69.
- Liston EH, Levine MD, Philippart M. Psychosis in Fabry disease and treatment with phenoxybenzamine. *Arch Gen Psychiatry* 1973; **29**: 402.

37. Rosenberg DM, Ferrans VJ, Fulmer JD *et al.* Chronic airflow obstruction in Fabry's disease. *Am J Med* 1980; **68**: 898.
38. Gemignani F, Pietrini Y, Tagliavini F *et al.* Fabry's disease with familial lymphedema of the lower limbs. *Eur Neurol* 1979; **18**: 84.
39. Funderburk SJ, Philippart M, Dale G *et al.* Priapism after phenoxybenzamine in a patient with Fabry's disease. *N Engl J Med* 1974; **290**: 630.
40. Whybra C, Kampmann CO, Willers I *et al.* Anderson-Fabry disease: clinical manifestations of disease in female heterozygotes. *J Inher Metab Dis* 2001; **24**: 715.
41. MacDermot KD, Homes A, Miners AH. Anderson-Fabry disease: clinical manifestations and impact of disease in a cohort of 60 obligate carrier females. *J Med Genet* 2001; **38**: 769.
42. Kampmann C, Baehner F, Whybra C *et al.* Cardiac manifestations of Anderson-Fabry disease in heterozygous females. *J Am Coll Cardiol* 2002; **40**: 1668.
43. Pompen AWM, Ruiter M, Wyers JGG. Angiokeratoma corporis diffusum (universale) Fabry, as a sign of an unknown internal disease: two autopsy reports. *Acta Med Scand* 1947; **128**: 234.
44. Scriba K. Zur Pathogenese des Angiokeratoma corporis diffusum Fabry mit cardiovasorenalem Symptomenkomplex. *Verh Deutsch Ges Pathol* 1950; **34**: 221.
45. Schibanoff JM, Kamoshita S, O'Brien JS. Tissue distribution of glycosphingolipids in a case of Fabry's disease. *J Lipid Res* 1969; **10**: 515.
46. Christenson-Lou HO. A biochemical investigation of angiokeratoma corporis diffusum. *Acta Pathol Microbiol Scand* 1966; **68**: 332.
47. Wherret JR, Hakimori S. Characterization of a blood group B glycolipid accumulating in the pancreas of a patient with Fabry's disease. *J Biol Chem* 1973; **248**: 3046.
48. Romeo G, Urso M, Pisacane A *et al.* Residual activity of α -galactosidase A in Fabry's disease. *Biochem Genet* 1975; **13**: 615.
49. Beutler E, Kuhl W. Purification and properties of human alpha-galactosidases. *J Biol Chem* 1972; **247**: 7195.
50. Beutler E, Kuhl W. Absence of cross-reactive antigen in Fabry disease. *N Engl J Med* 1973; **289**: 694.
51. Lemansky P, Bishop DF, Desnick RJ *et al.* Synthesis and processing of α -galactosidase A in human fibroblasts. Evidence for different mutations in Fabry disease. *J Biol Chem* 1987; **262**: 2062.
52. Romeo G, Migeon BR. Genetic inactivation of the alpha-galactosidase locus in carriers of Fabry's disease. *Science* 1970; **170**: 180.
53. Calhoun DH, Bishop DF, Bernstein HS *et al.* Fabry disease: isolation of a cDNA clone encoding human alpha-galactosidase A. *Proc Natl Acad Sci USA* 1985; **82**: 7364.
54. Bernstein HS, Bishop DF, Astrin KH *et al.* Fabry disease: gene rearrangements and a coding region point mutation in the alpha-galactosidase A gene. *J Clin Invest* 1989; **83**: 1390.
55. Eng CM, Resnick-Silverman LA, Niehaus DJ *et al.* Nature and frequency of mutations in the alpha-galactosidase A gene causing Fabry disease. *Am J Hum Genet* 1993; **53**: 1186.
56. Eng CM, Desnick RJ. Molecular basis of Fabry disease: mutations and polymorphisms in the human α -galactosidase A gene. *Hum Mutat* 1994; **3**: 103.
57. Eng CM, Ashley GA, Burgert TS *et al.* Fabry disease: thirty-five mutations in the α -galactosidase A gene in patients with classic and variant phenotypes. *Mol Med* 1997; **3**: 174.
58. Yasuda M, Shabbeer J, Osawa M, Desnick RJ. Fabry disease: novel α -galactosidase A 3'-terminal mutations result in multiple transcripts due to aberrant 3'-end formation. *Am J Hum Genet* 2003; **73**: 162.
59. Beaudet A, Caskey CT. Detection of Fabry's disease heterozygotes by hair root analysis. *Clin Genet* 1978; **13**: 251.
60. Brady RO, Uhlenhuth BW, Jacobson CB. Fabry's disease: antenatal diagnosis. *Science* 1971; **172**: 174.
61. Galjaard H, Niermeijer MF, Hahnenmann N *et al.* An example of rapid prenatal diagnosis of Fabry's disease using microtechniques. *Clin Genet* 1974; **5**: 368.
62. Kleijer WJ, Hussaarts-Odijk LM, Sacks ES *et al.* Prenatal diagnosis of Fabry's disease by direct analysis of chorionic villi. *Prenat Diagn* 1987; **7**: 283.
63. Lenoir G, Rivron M, Gubler MC *et al.* La maladie de Fabry. Traitement du syndrome acrodyniforme par la carbamazepine. *Arch Fr Pediatr* 1977; **34**: 704.
64. Germain DP. Fabry's disease (alpha-galactosidase-A deficiency): recent therapeutic innovations. *J Soc Biol* 2002; **196**: 183.
65. Inagaki M, Ohno K, Ohta S *et al.* Relief of chronic burning pain in Fabry disease with neurotrophin. *Pediatr Neurol* 1990; **6**: 211.
66. Clarke JTR, Guttman RD, Wolfe LS *et al.* Enzyme replacement therapy for renal allotransplantation in Fabry's disease. *N Engl J Med* 1972; **287**: 1215.
67. Schweitzer EJ, Drachenberg CB, Bartlett ST. Living kidney donor and recipient evaluation in Fabry's disease. *Transplantation* 1992; **54**: 924.
68. Desnick RJ, Dean KJ, Grabowski GA *et al.* Enzyme therapy XII: enzyme therapy in Fabry's disease – differential enzyme and substrate clearance kinetics of plasma and splenic alpha-galactosidase isozymes. *Proc Natl Acad Sci USA* 1979; **76**: 5326.
69. Friedlaender MM, Kopolovic J, Rubinger D *et al.* Renal biopsy in Fabry's disease eight years after successful renal transplantation. *Clin Nephrol* 1987; **27**: 206.
70. Desnick RJ, Brady R, Barranger J *et al.* Fabry disease an under-recognized multisystemic disorder: expert recommendations for diagnosis management and enzyme replacement therapy. *Ann Int Med* 2003; **138**: 338.
71. Schiffmann R, Kopp JB, Austin HA III *et al.* Enzyme replacement therapy in Fabry disease. *J Am Med Assoc* 2001; **27**: 43.
72. Wilcox WR, Banikazemi M, Guffon N *et al.* Long-term safety and efficacy of replacement therapy for Fabry disease. *Am J Hum Genet* 2004; **75**: 65.
73. Blom D, Speijer D, Linthorst GE *et al.* Recombinant enzyme therapy for Fabry disease: absence of editing of human α -galactosidase A mRNA. *Am J Hum Genet* 2003; **72**: 23.

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74. Ogawa Y, Nanba E, Ohno K *et al.* A new therapeutic approach to β -galactosidosis: galactose analog compounds of low molecular weight restore mutant human β -galactosidases expressed in enzyme-deficient knockout mouse fibroblasts. *Proc Jpn Soc Inherit Metabol Dis* 2001; **44**: 238.
75. Asano N, Ishii S, Kizu H *et al.* *In vitro* inhibition and intracellular enhancement of lysosomal α -galactosidase A activity in Fabry lymphoblasts by 1-deoxygalactonojirimycin and its derivatives. *Eur J Biochem* 2000; **267**: 4179.
76. Abe A, Gregory S, Lee L *et al.* Reduction of globotriaosylceramide in Fabry disease mice by substrate deprivation. *J Clin Invest* 2000; **105**: 1563.

GM₁ gangliosidosis/ β -galactosidase deficiency

Introduction	666	Treatment	674
Clinical abnormalities	667	References	674
Genetics and pathogenesis	671		

MAJOR PHENOTYPIC EXPRESSION

Cerebral degenerative disease of infantile onset combining features of a mucopolysaccharidosis including dysostosis multiplex and hepatosplenomegaly with those of a neurolipidosis, including cherry red macular spots; accumulation of the ganglioside GM₁ in the brain and viscera and of mucopolysaccharides in viscera; and deficiency of acid β -galactosidase. Variants with late infantile, juvenile, or adult forms have progressive neurologic degeneration. Defects in same enzyme cause Morquio B disease, in which there is a mild Morquio phenotype.

INTRODUCTION

Infantile GM₁ or generalized gangliosidosis is a lysosomal storage disease in which GM₁ ganglioside (Figure 89.1) accumulates in the brain and viscera [1–5]. The resultant cerebral degenerative disease is a devastating one, and affected patients usually die before two years of age. This was the first of the GM₁ gangliosidoses to be described [5], and it is the most common. It has also been referred to as type 1, but as increasing genetically determined

variation becomes evident, it is less appropriate to number these disorders. A spectrum of considerable differences in phenotype appears to reflect different degrees of residual enzyme activity resulting from different mutations. It has been practical clinically to consider GM₁ gangliosidosis broadly as infantile, juvenile, or adult.

Defective activity of β -galactosidase (EC 3.2.1.23) (Figure 89.1) was first discovered by Okada and O'Brien [6]. Recognition of the enzyme abnormality led to the elucidation of later onset forms of GM₁ gangliosidosis [7–9]. Spondyloepiphyseal dysplasia and normal intelligence in a Morquio-like syndrome were also found in patients with deficiency of the same β -galactosidase enzyme [10–12].

The multienzyme complex with cathepsin A includes β -galactosidase and N-acetylaminogalacto-6-sulfate-sulfatase and sialidase. The genes are, respectively, NEU1; cathepsin A and PPGB; and GLB. Mutations in any of these components lead to functional deficiency and severe lysosomal storage disease [13]. GM₁ gangliosidosis and Morquio type B have an incidence of about 1:100,000–1:200,000 live births [14].

The gene for β -galactosidase has been assigned to chromosome 3p21.33 [15]. The human placental cDNA was cloned by Oshima *et al.* [16], and found to have a coding sequence of 2031 nucleotides encoding a protein of 677 amino acids. A number of mutations has been found in GM₁ gangliosidosis [17, 18], and in Morquio B disease [19, 20], and these very different phenotypes are allelic.

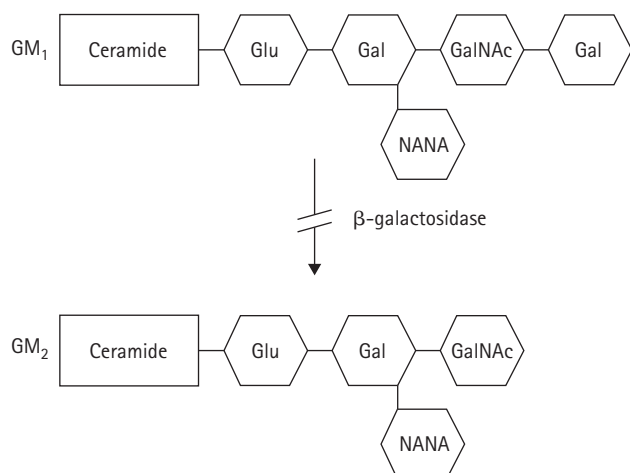


Figure 89.1 Lysosomal acid β -galactosidase. The site of the defect in GM₁ gangliosidosis.

CLINICAL ABNORMALITIES

Infantile GM₁ gangliosidosis

Classic GM₁ gangliosidosis differs from most of the other storage diseases in that abnormalities are present from birth. The first symptoms are facial edema, pitting edema of the extremities or ascites, and motor impairment, evident in poor sucking and appetite and failure to thrive. Some infants present with hydrops fetalis [21, 22]. In a patient with transient fetal ascites, examination of the macroscopically normal placenta revealed vacuolated cells, and enzyme analysis confirmed the diagnosis of GM₁ gangliosidosis. The infant is usually hypotonic and hypoactive. The facial features may be coarse, even very early, and the expression is dull. There is frontal bossing, downy hirsutism over the forehead, a depressed nasal bridge, large, low-set ears, and an increased distance between the nose and the upper lip (Figures 89.2, 89.3 and 89.4). Patients have hypertrophy of the gums and a large tongue [3–5]. A cherry red macular spot (Figure 89.5) is visible bilaterally in about half the patients [23]. Nystagmus may be present. The cornea may show mild or absent clouding, but a true cloudy cornea has been reported in one patient [24]. Hepatomegaly is prominent, and the spleen may be palpable.

By eight months of age the infant may be able to hold up his head, but he cannot sit or crawl. He may follow objects with his eyes and even reach for them, but his grasp is poor. Movements are uncoordinated. The infant rarely smiles

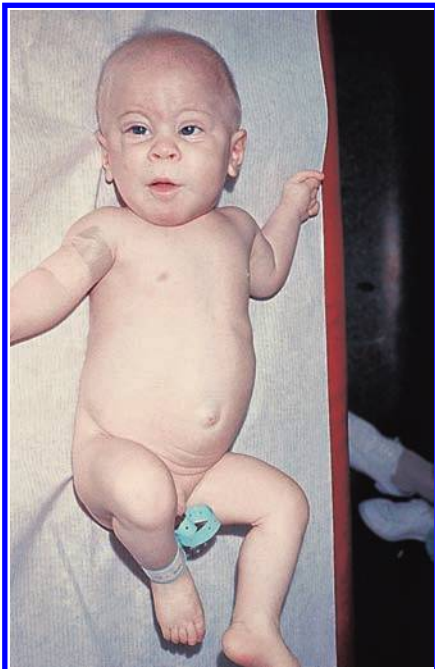


Figure 89.2 CD: A four-month-old infant with generalized GM₁ gangliosidosis. Features were coarse. Frontal bossing, a depressed nasal bridge, and low-set ears can be seen. (Courtesy of Dr John S O'Brien, University of California San Diego.)

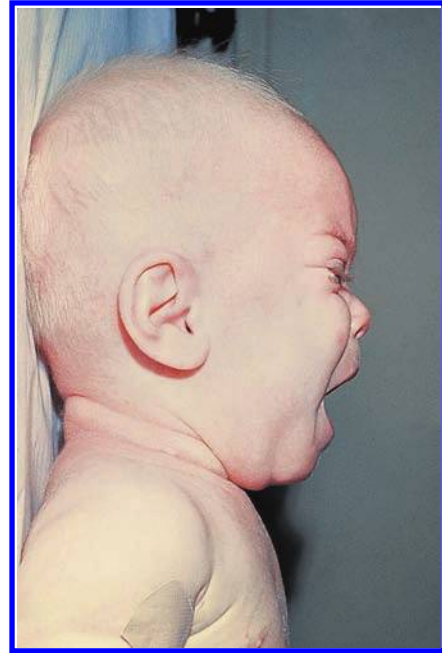


Figure 89.3 CD: Lateral view of the face illustrates the coarse features. The ear is low-set and the nasal bridge depressed. The prominent maxillary area is characteristic. (Courtesy of Dr John S. O'Brien, University of California San Diego.)



Figure 89.4 A four-month-old Japanese baby with GM₁ gangliosidosis. Features were a little coarse. There was frontal bossing and a depressed nasal bridge.

and appears uninterested in his environment. His cry is weak. Parents may say he is a good baby, meaning that he sleeps a lot and is immobile much of the day. Hepatomegaly is uniformly found after six months, and splenomegaly



Figure 89.5 Cherry red macular spot in an infant with GM₁ gangliosidosis.

is seen in 80 percent of patients. Coarse features are accentuated. Macrocephaly may develop, but it is less frequent and less prominent than in Tay–Sachs disease. Deep tendon reflexes become hyperactive, and hyperacusis may develop [3, 25]. Muscle weakness progresses, and there may be a head lag on elevating the shoulders. Early hypotonia is replaced by spasticity. Deep tendon reflexes are exaggerated, and pyramidal tract signs are evident. After the first year, deterioration is rapid. Convulsions are frequent, and swallowing is so poor that tube feeding is required. Recurrent pneumonias complicate the course. By 16 months the patient is blind and deaf and has decerebrate rigidity. Optic atrophy is seen and the retina may be edematous [26]. Flexion contractures develop, and there is no response to stimuli. Death usually follows pneumonia by two years of age. Angiokeratomas have been described in this disease as early as ten months [27]. We have seen a patient with a single lesion on a leg. They may be widely scattered, but do not cluster as in Fabry disease, and they have not been observed on the penis or scrotum.

An unusual presentation has been observed in infants with GM₁ gangliosidosis [28–30], suggesting that this disorder should be included in the differential diagnosis of cardiomyopathy. Each developed congestive cardiac failure. Awareness that this disorder may present in this fashion should facilitate early diagnosis in such patients.

The skeletal manifestations of the disease are also progressive. The fingers are short and stubby. The joints are stiff and limited in motion. There are flexion contractures of the fingers, especially the fifth. The wrists and ankles become enlarged. Flexion contractures also occur at the elbows and knees. The dorsolumbar kyphosis may be a prominent gibbus. The characteristic picture is that of a mucopolysaccharidosis, but in some patients these features may be subtle, and patients with severe neurodegeneration may not have recognizable dysmorphic features [31].

Roentgenographic findings are usually those of an early and very severe dysostosis multiplex. These changes are



Figure 89.6 CD: Roentgenograms of the arm at seven months. The picture was that of advanced dysostosis multiplex. The bones were short and had prominent midshaft thickening. The distal ends of the radius and ulna had begun to point toward each other. The distal end of the humerus showed the characteristic pinched-off appearance. The changes in the bones in this syndrome are just like those of Hurler syndrome, but in this disease they are prominent much earlier in life. (Courtesy of Dr John S O'Brien, University of California San Diego.)

similar to those of I-cell disease (Chapter 83), and they are usually earlier in onset and more severe than those of Hurler syndrome (Chapter 76) [3, 32]. The long tubular bones are shortened and widened in midshaft, tapering distally and proximally (Figure 89.6). Subperiosteal new bone formation is characteristic. The consequent cloaking of the already widened bones is particularly evident in the humerus, and the pinching-off of the end of the bones is striking. The distal ends of the radius and ulna tilt obliquely toward each other. Middle and proximal phalanges are widened. The metacarpals are short and broad and taper proximally. The carpal centers are hypoplastic. The lumbar vertebrae are hypoplastic and beaked anteriorly at the site of the kyphosis (Figure 89.7). The ribs are thickened and spatula-like. The ilia are flared. The sella turcica is shallow and elongated, giving it a shoe-shaped appearance. Bone age is impaired [33].

Neuroimaging may show white matter changes followed by loss of white matter and atrophy [34]. Increased signal has been seen in the basal ganglia [35], and calcification of the basal ganglia has been reported [8]. The electroencephalogram (EEG) may be normal early on [33], but later there is evidence of dysrhythmia [36, 37]. Visual evoked potentials are abnormal [36].

Neuronal lipidosis is prominent on histologic examination of the brain. The ballooned neurons look exactly like those of Tay–Sachs disease [38, 39]. Indeed, electron microscopy shows identical lamellar cytoplasmic inclusion bodies in the neurons. These membranous cytoplasmic bodies also may be seen in retinal ganglion cells [23]. In viscera such as the liver there is histiocytosis, and vacuoles may be present in both hepatocytes and histiocytes that



Figure 89.7 CD: Roentgenogram of the spine at seven months. The vertebral bodies were hypoplastic. There was anterior breaking of L1 and L2. (Courtesy of Dr John S O'Brien, University of California San Diego.)



Figure 89.8 Histiocytic foam cell in the marrow of a patient with GM₁ gangliosidosis.

stain with periodic acid-Schiff. A characteristic lesion is the cytoplasmic vacuolation and ballooning of the renal glomerular epithelial cells [4], seen only in this disorder and in Fabry disease ([Chapter 88](#)). Lysosomal inclusions in the epithelial cells of the skin may aid in the diagnosis [40]. There are vacuolated lymphocytes in the peripheral blood and foamy histiocytes in the bone marrow ([Figure 89.8](#)).

Mucopolysacchariduria usually has not been detected, but keratan sulfate has been reported in excess in the urine [39]. The ganglioside that accumulates in the brain and in the viscera in generalized gangliosidosis

is the GM₁ ganglioside [3, 5, 41]. It is the major monosialoganglioside of normal brain. Its structure is galactosyl(1→3)-N-acetylgalactosaminyl-(1→4)-[(2→3)-N-acetylneuraminyl]-galactosyl-(1)-glycosyl-(1→1)-[2-N-acyl]-sphingosine. Thus, it is a trihexoside with an N-acetylneuraminic acid side chain. It differs from the Tay-Sachs, or GM₂, ganglioside in the presence of the terminal galactose on GM₁. This ganglioside is present in ten times normal amounts in the cerebral gray matter of patients with GM₁ gangliosidosis. Mucopolysaccharides also are stored in peripheral tissues [41]. The magnitude of the storage is similar to that in Hurler disease, but the mucopolysaccharides stored differ, in that they more closely resemble keratansulfate. It appears reasonable that the abnormalities of bone are caused by the mucopolysaccharide storage and that cerebral degeneration is a consequence of the storage of ganglioside in the brain.

Other genetic variants

LATE INFANTILE/JUVENILE GM₁ GANGLIOSIDOSIS

GM₁ ganglioside also accumulates in the brain in patients with onset in late infancy or childhood [38, 42–45]. In chronic adult GM₁ gangliosidosis it is found only in the caudate and the putamen [46].

Patients with the late infantile/juvenile disease have progressive cerebral deterioration, but it begins later than in the classic form, often at about one year of age [47]. Onset may be with ataxia. There may be incoordination or frequent falling and generalized muscular weakness. Speech, if present, is lost. Thereafter, mental and motor deterioration may progress rapidly. Patients develop spasticity and rigidity, and they have seizures, which may be a major problem in management. They exhibit myoclonus, sound-induced myoclonus and myoclonic seizures [45]. They usually die between three and seven years of age in a state of decerebrate rigidity.

Phenotypic expression may be quite variable in these patients. The facial appearance may be coarse in late infantile patients. It may be quite normal in juvenile patients ([Figures 89.9, 89.10, 89.11, 89.12, 89.13](#) and [89.14](#)). Similarly, hepatosplenomegaly may be present ([Figure 89.12](#)), but is usually absent [46]. There may be internal strabismus or nystagmus. There are usually no cherry red spots or other fundoscopic findings, although blindness may occur later; and atypical cherry red spots were reported in one patient [48]. Many widely disseminated Mongolian spots were seen in one patient ([Figures 89.11](#) and [89.12](#)). Extensive Mongolian spots have also been reported in some infantile patients [49–51].

EEG may be abnormally slow or show spike discharges. Neuroimaging reveals cerebral atrophy [45]. Patients have vacuolated cells in the bone marrow. Sea blue histiocytes have been reported [45]. Membranous cytoplasmic



Figure 89.9 MS: A nine-year-old Saudi girl with juvenile GM₁ gangliosidosis. She is shown in an essentially decerebrate posture. Early development was normal, and she walked at less than one year, but shortly thereafter began to lose milestones. Seizures began at four years. There were no coarse or dysmorphic features in contrast to classic GM₁ gangliosidosis. There was no dystosis multiplex. β -Galactosidase activity in fibroblasts was 1.7 percent of control.



Figure 89.10 MS: The legs were in a tonic neck response. They were very spastic and deep tendon reflexes were exaggerated. Plantar reflexes were down. There were vacuolated histiocytes in the marrow.

bodies are found in the neuronal cytoplasm, and large amounts of GM₁ ganglioside accumulate in the brain [42]. Keratosulfaturia has been described [42].

CHRONIC/ADULT GM₁ GANGLIOSIDOSIS

The adult form of GM₁ gangliosidosis storage disease presents with progressive cerebellar dysarthria, progressive ataxia, myoclonus, and spasticity [9, 52, 53]. Dystonia is a major manifestation in many patients [54–56]. Intellectual impairment may be mild, but there is usually loss of function over time. There may be abnormalities of gait or speech. These patients do not usually have cherry red spots. Rare patients have cherry red spots and progressive disease



Figure 89.11 AMAQ: The hyperpigmented areas appeared to be diffuse Mongolian spots.



Figure 89.12 AMAQ: Hepatosplenomegaly. The pigmentation was also present over the abdomen.

[57]. They do not have dysmorphic features, suggesting storage of mucopolysaccharide. In one patient, onset was at 2–3 years of age with unsteadiness of gait, and at five years he was thought to have cerebral palsy. Dystonia began at six years and was extreme by 15. He died at 27 years of age. Pathologic changes were largely confined to the basal ganglia, where there was intraneuronal storage and accumulation of GM₁ ganglioside [56]. Seizures



Figure 89.13 AS: The seven-year-old brother of MS also had juvenile GM₁ gangliosidosis. He lay in bed in decerebrate posture with rigid arms and legs. He showed no interest in his surroundings, but was conscious. There was no coarsening of the features.



Figure 89.15 JAH: An 11-year-old boy with Morquio type B disease. He and his affected brother were severely mentally impaired. Facial features were coarse. (This illustration was kindly provided by Dr Philip Benson.)



Figure 89.14 AS: The legs were spastic in flexion and extension and deep tendon reflexes were accentuated.

are uncommon, and vision is preserved. Kyphosis and moderate flattening of vertebral bodies has been reported [53] as well as flattening of the femoral heads.

MORQUIO B DISEASE

A very different clinical phenotype has been described in which there is severe skeletal dysplasia without neurologic involvement [11, 58, 59] (Figure 89.15). This condition is also referred to as mucopolysaccharidosis IV B, as well as Morquio syndrome type B [11, 60]. One patient presented

at 20 years of age with pain in the hip and was found to have progressive dysplasia of the pelvis and the femoral heads [58]. She had vertebral platyspondyly and modeling abnormalities of the vertebral bodies, like those seen in hereditary spondyloepiphyseal dysplasia. A different variant was reported [60] in which a brother and sister had typical clinical and roentgenographic features of Morquio disease, but had atypical mental regression. In these patients a severe deficiency was found in the activity of β -galactosidase in cultured fibroblasts and in leukocytes.

GENETICS AND PATHOGENESIS

GM₁ gangliosidosis and all of the β -galactosidase variants are transmitted in an autosomal recessive fashion. In any family in which multiple patients are seen, each one virtually always has the same phenotype. However, phenotypic variation within a family has been reported [61]. A risk of recurrence of 25 percent in siblings is consistent with observed data [2, 3]. The rate of consanguinity in reported families has been high. There is no ethnic predominance for the common classic infantile forms, or most of the other variants, but the adult form has been reported predominantly from Japan.

Detection of the heterozygous carrier has been accomplished by the assay of the enzyme in leukocytes [62], and heterozygotes as a group can be distinguished from normals by enzyme assay in a statistically significant fashion;

however, overlap with normals makes detection potentially unreliable in any individual in which a normal value is obtained. When the mutation is known, carrier detection is highly reliable [54]. In adult type Japanese families the I51T mutation has been useful for this purpose [54].

Intrauterine diagnosis by assay of β -galactosidase in cultured amniocytes or chorionic villus cells is an established procedure [63–65]. Rapid prenatal diagnosis has been accomplished by the direct analysis of galactosyloligosaccharides in amniotic fluid using high performance liquid chromatography [66] and rapid enzymatic diagnosis with chromosome microarray assay [67]. Preimplantation genetic diagnosis has been reported [68] with chromosome microarray for aneuploidy coupled with testing for specific DNA sequences for GM₁ gangliosidosis mutations in a couple who were carriers for GM₁ gangliosidosis and was carried out by trophectoderm biopsy followed *in vitro* fertilization.

The mutations causing each type of GM₁ gangliosidosis are allelic. Somatic cell hybridization has failed to reveal evidence of complementation [69]. The fundamental defect in all of the GM₁ gangliosidoses is the nearly complete activity of the lysosomal acid β -galactosidase [6]. The enzyme is synthesized as an 8 kD protein which is processed to a 64 kD monomeric protein which contains 7.5 percent carbohydrate [70, 71].

The precursor protein is coded for by a gene on chromosome 3 (3p21.33) [13, 72, 73]. The 64 kDa monomer aggregates to a homopolymer of about 700 kDa, a process that is promoted by association with a 32 kDa protein coded for by a gene originally mapped to chromosome 22 [74] which increases stability in the presence of proteases. It is this 32 kDa protein that is the defect in galactosialidosis, protective protein/cathepsin A (PPCA), and it is actually located on chromosome 22q13.1 [75] (Chapter 100). Different forms of β -galactosidase have been separated electrophoretically or isolated, representing monomer, dimer, and multimer. The enzyme hydrolyzes the terminal galactose of GM₁ (Figure 89.1). It also cleaves

a terminal galactose from asialoGM₁, lactosylceramide, keratan sulfate, lactose, and a variety of oligosaccharides. It does not cleave galactocerebroside, which is cleaved by galactocerebroside β -galactosidase (EC 3.2.1.46), the enzyme that is deficient in Krabbe disease (Chapter 96).

In GM₁ gangliosidosis, the deficiency of the enzyme may be demonstrated using an artificial substrate such as *p*-nitrophenylgalactoside and 4-methylumbelliferyl-galactoside. It also may be done using isolated GM₁ ganglioside. The activity of this enzyme is markedly deficient when GM₁ ganglioside or galactose-containing glycoprotein is used as substrate [75, 76]. The enzyme is markedly deficient in liver and brain [69] and virtually inactive in leukocytes [77] or cultured fibroblasts [78]. GM₁ ganglioside β -galactosidase activities have been reported as less than 0.1 percent of normal [67]. Normal amounts of immuno-cross-reactive material have been found [75]. The activity of residual enzyme is not different in infantile and juvenile forms of GM₁ gangliosidosis [42]. Patients with juvenile GM₁ gangliosidosis have considerably more activity when tested against GM₁ ganglioside [6] than patients with the infantile disease. Higher levels, 5–10 percent, of control activity have been reported in adult GM₁ gangliosidosis [16, 54]. Impaired degradation of keratan sulfate from shark cartilage was shown to be deficient in fibroblasts of patients with GM₁ gangliosidosis [79]. In patients with Morquio B disease, activities of 5–10 percent [58] and less than 5 percent [80] have been reported with actually varying activity against varying substrates. Activity against keratan sulfate was undetectable in these patients [12]. Qualitative differences in pH optima and in thermolability have been observed among variants [81].

β -Galactosidase splits the terminal galactose from GM₁ converting it to GM₂. It also cleaves galactose [76] from the mucopolysaccharides that accumulate in the viscera of patients. Thus, the storage of both ganglioside and mucopolysaccharide may be seen as direct consequences of the deficiency of the galactosidase.

The pleiotropic effect of the single mutation that gives

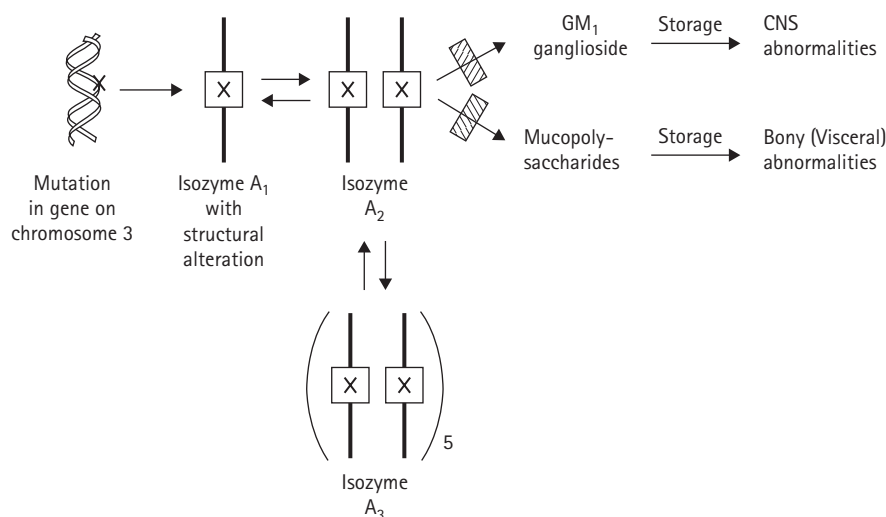


Figure 89.16 Model of the pathogenesis of the phenotypes of GM₁ gangliosidosis. The phenotypic effect of a single gene mutation results from the effects of a single enzyme on multiple substrates.

rise to the GM₁ gangliosidosis phenotype conforms to a one gene one polypeptide enzyme multiple substrates model (Figure 89.16). The A₁ enzyme is a monomer that spontaneously dimerizes to form A₂, which can reversibly be formed from the A₃ [70, 75]. The A₁ isozyme is heterocatalytic, cleaving galactose from many galacto-conjugates, among which the GM₁ ganglioside and mucopolysaccharide are two. Different phenotypes in which bone or cerebral manifestations predominate could result from different mutations that selectively affect the catalytic activity for one or another set of substrates. Patients have been described [58] in whom there is relatively normal activity against the peripheral galactose-containing glycoconjugates and little or none against GM₁, and the phenotype is of rapid neurologic degeneration without bony abnormalities. Similarly, patients with the Morquio type B phenotype have severe skeletal dysplasia without neurologic abnormalities [11, 58, 59].

The direct consequences of the defective enzymatic activity against the various substrates are the accumulation of ganglioside in brain and keratan sulfate in bone and tissues. In addition, galactose-containing oligosaccharides accumulate in the viscera and are excreted in the urine, which can be very useful in diagnosis [14–19, 23, 32, 42, 70, 82–87]. The variety of oligosaccharides excreted is great. The most abundant are similar to partially degraded erythrocyte glycoprotein [70]. They are all galacto-oligosaccharides that contain a terminal galactose in β -1-4 linkage to N-acetylglucosamine [83, 87]. This is in contrast to GM₁ ganglioside, in which the linkage of the terminal galactose is 1→3 to N-acetylgalactosamine. In the most sensitive method [86], which employs the high performance liquid chromatography of oligosaccharides made radioactive by reaction with tritium-labeled sodium borohydride, the infantile phenotype could be distinguished from the juvenile by an increase of three- to ten-fold higher levels of oligosaccharides and especially higher concentrations in the higher molecular weight compounds. Patients with adult onset disease had levels 130- to 180-fold lower than those with infantile onset disease and no high molecular weight oligosaccharides. Three fractions were present in the infantile but not in the juvenile patients, suggesting an ability of the juvenile patients to cleave compounds not cleavable in the infantile form [85].

The inner membrane of the nuclear envelope (NE) of neurons and other cells in GM₁ is tightly associated with a Na(+)/Ca(2+) exchanger (NCX). It is involved in transfer of Ca(2+) ions from nucleoplasm to the NE lumen. The NCX/GM1 complex appears to be located in the inner membrane of the NE. Cultured neurons from knockout mice lacking GM₁ synthase were vulnerable to Ca(2+)-induced apoptosis. These neurons in culture were partially rescued by GM₁ and more effectively by LIGA-20, a membrane-permeant derivative of GM₁NE [88].

In a mouse model of GM₁ gangliosidosis, the GM₁ ganglioside was found to accumulate in the glycosphingolipid-enriched microdomain (GEM) of the

mitochondria-associated endoplasmic reticulum (ER) membranes and to interact with the phosphorylated receptor, influencing calcium flux and moving calcium from the ER to the mitochondria. This opens the permeability transition pore, activating mitochondrial apoptosis [89].

The cloning of the human gene for β -galactosidase [16] permitted elucidation of the molecular biology of the β -galactosidase deficiencies. The gene contains 16 exons. Mutations have been found in examples of each of the clinical phenotypes. Most have been missense mutations, and the majority of patients studied have been Japanese [16–18, 54] (Table 89.1). In general, mRNA is normal in size and quantity in late infantile, adult, and Morquio B phenotypes, and reduced or absent in infantile patients. In an infantile patient with reduced but detectable mRNA, a point mutation led to a change from arginine 49 to cysteine (R49C) [17]. In another infantile patient arginine 457 was changed to a stop codon [17]. Among these patients, two duplications were found [18, 19, 90]; that in exon 3 led to a premature termination. The other led to an abnormally large mRNA. R208C has been found relatively commonly in American infantile patients [91], and R482H in Italian patients with this phenotype [92]. In four juvenile Japanese patients, all were found to have a mutation of arginine

Table 89.1 Some mutations in the gene for β -galactosidase

Exon	Amino acid	DNA
2	Arg49 → Cys	CGC → TGC
3	Gly123 → Arg	GGG → AGG
3	Duplication-stop codon	288–310
6	Arg201 → Cys	CGG → TGC
	Arg208 → Cys	CGG → TGC
9	Tyr316 → Cys	TAT → TGT
11–12	Duplication	1103–1267
14	Arg457 → Ter	CGA → TGA
	Arg482 → His	CGE → CAC
15	Gly494 → Cys	GGT → TGT
15	Lys577 → Arg	AAG → AGG
Late infantile/juvenile GM₁ gangliosidosis		
6	Arg201 → Cys	GCG → TGC
16	Glu632 → Gly	GAA → GGA
Chronic adult GM₁ gangliosidosis		
2	Ile51 → Thr	ATC → ACC
2–3	Thr82 → Met	ACG → ATG
14	Arg457 → Gln	CGA → CAA
Morquio B disease		
8	Trp273 → Leu	TGG → CTG
14	Arg482 → Cys	CGC → CGT
15	Trp509 → Cys	TGG → TGT

201 to cystine [17], while six adult patients all had the isoleucine 51 to threonine mutation (I51T). This mutation [17] creates a Bsu36I restriction site in exon 2 that is useful for diagnosis and genetic analysis [54]. Some compounds of two mutant genes have been observed. In Morquio B disease, three missense mutations have been identified; all three individuals in two families were genetic compounds [17]. W273L has been relatively common in Caucasian patients with the Morquio B phenotype [19]. In general, among compounds, the allele with the higher residual activity determines the clinical phenotype [91].

Compound heterozygosity for p.G494V and c.495-497-delTCT mutations were found in Chinese patients with cardiomyopathy and the infantile disease [93]. Missense mutations, p.H102 D and p.A301V, were found in patients with the juvenile form. As many as 102 mutations have been found to be distributed along the β -galactosidase gene (GLB1) [94]. In 21 unrelated patients with GM₁ gangliosidosis and four Morquio B patients, 27 mutations were found, nine of them novel; there were five missense, three microdeletions, and a nonsense mutation [14]. In Portuguese patients, six different mutations were found in the GLB1 gene, all of them previously described (p.R59H, p.R201H, p.H281Y, p.W527X, c.1572-1577InsG, and c.845-846delC) [13]. In 16 patients with a spectrum of phenotypes, 28 mutations were found including: p.I55FfsX16, p.W65X, p.F107L, p.H112P, p.C127Y, p.W161X, p.I181K, p.C230R, p.W273X, p.R299VfsX5, p.A301V, p.F357L, p.K359KfsX23, p.L389P, p.D448V, p.D448GfsX8, and the intronic mutation IVS6-8A>G [95].

TREATMENT

Effective treatment has not yet been developed. The availability of animal models, in both cats [96] and dogs [97], as well as sheep and cattle, provides subjects for the exploration of experimental approaches to therapy. Allogenic bone marrow transplantation in an affected Portuguese water dog was without effect on clinical course or enzyme activity despite engraftment. Amniocyte transplantation was without effect in a patient with Morquio B disease. Trihexyphenidyl appeared to modulate dystonia in a patient with the adult phenotype [98].

Gene transfer is under study in experimental animals. Enzyme activity was enhanced in GM₁ mice injected into the thalamus and deep cerebellar nuclei with an adenovirus- β -galactosidase vector. Storage of glycosphingolipids was reduced to almost normal in spinal cord. Survival was significantly longer than in untreated mice but motor performance declined in similar fashion to that of untreated animals [99]. Among promising approaches to therapy, substrate reduction with the iminosugar N-butyldeoxygalactonojirimycin (Zavesca®) has been shown [100] to significantly reduce cerebral storage of GM₁ ganglioside in β -galactosidase-deficient knockout mice. Another approach is chaperone therapy: N-octyl-

4-epi-b-valienamine (NOEV) is an inhibitor of lysosomal β -galactosidase, but in low concentration it restored enzyme activity in cultured human fibroblasts expressing the mutations R201C and R201H [101]. In addition, oral administration for a week enhanced enzyme activity in the brain of a mouse model of juvenile GM₁ gangliosidosis expressing the R201C mutant protein and reduced histochemically detected GM₁ in brain. It is thought that the effects of NOEV represent chaperone function which would be expected to function in the case of milder variants whose enzyme is synthesized with normal or near normal enzyme activity, but fails in normal transport to the lysosome. In GM₁-gangliosidosis model mice, oral NOEV was found to enter the brain and to increase β -galactosidase activity and reduce substrate storage [102]. The iminosugar 1-deoxygalactonojirimycin had been found to have effects as a pharmacologic chaperone. It is a competitive inhibitor of the enzyme and protects it from degradation. A variety of mutant alleles including p.R201C, p.R201H, p.C230R, and p.G438E were found to have significant sensitivity and improved transport and lysosomal processing [103]. Iminoalditol type glycosidase inhibitors and perfluorinated N-substituents have also been found to exert pharmacological chaperone enhancement of the activities of mutants associated with GM₁ gangliosidosis and Morquio B disease [104]. In further approaches to chemical modifications of 1-deoxygalactonojirimycin N-alkylation of the ring nitrogen followed by aromatic substitution has produced lipophilic derivatives which inhibit β -glycosidases of microorganisms and plant origin. They may serve as pharmacologic chaperones in specific GM₁-gangliosidosis mutant enzymes [105].

REFERENCES

- O'Brien JS. Ganglioside storage diseases. An updated review. *J Neurol Sci* 1981; **3**: 219.
- Sandhoff K, Christomanou H. Biochemistry and genetics of gangliosidoses. *Hum Genet* 1979; **50**: 107.
- O'Brien J. Generalized gangliosidosis. *J Pediatr* 1969; **75**: 167.
- Landing BH, Silverman FN, Craig JM et al. Familial neurovisceral lipidosis. *Am J Dis Child* 1964; **108**: 503.
- O'Brien JS, Stern MB, Landing BH et al. Generalized gangliosidosis. *Am J Dis Child* 1965; **109**: 338.
- Okada S, O'Brien JS. Generalized gangliosidosis: beta-galactosidase deficiency. *Science* 1968; **160**: 1002.
- O'Brien JS, Ho MW, Veath ML et al. Juvenile GM₁-gangliosidosis: clinical, pathological, chemical and enzymatic studies. *Clin Genet* 1972; **3**: 411.
- Lowden JA, Callahan JW, Norman MG. Juvenile GM₁-gangliosidosis. Occurrence with absence of two beta-galactosidase components. *Arch Neurol* 1974; **31**: 200.
- Suzuki Y, Nakamura N, Fukuoka K et al. β -Galactosidase deficiency in juvenile and adult patients. Report of six Japanese cases and review of literature. *Hum Genet* 1977; **36**: 219.

10. O'Brien JS, Gugler E, Giedion A *et al*. Spondyloepiphyseal dysplasia, corneal clouding, normal intelligence and acid β -galactosidase deficiency. *Clin Genet* 1976; **9**: 495.
11. Arbisser AI, Donnelly KA, Scott CI *et al*. Morquio-like syndrome with β -galactosidase deficiency and normal hexosamine sulfatase activity: mucopolysaccharidosis IVB. *Am J Med Genet* 1977; **1**: 195.
12. van der Horst GTJ, Kleijer WJ, Hoofgeveen AT *et al*. Morquio B syndrome: a primary defect in β -galactosidase. *Am J Med Genet* 1983; **16**: 261.
13. Coutinho M, Lacerda L, Macedo-Ribeiro S *et al*. Lysosomal multienzymatic complex-related diseases: a genetic study among Portuguese patients. *Clin Genet* 2011 Jan 10. doi: 10.1111/j.1399-0004.2011.01625.x. [Epub ahead of print]
14. Caciotti A, Garman SC, Rivera-Colón Y *et al*. GM1 gangliosidosis and Morquio B disease: An update on genetic alterations and clinical findings. *Biochim Biophys Acta* 2011 Apr 7. [Epub ahead of print]
15. Shows TB, Scrafford-Wolff L, Brown JA, Meisler M. Assignment of a beta-galactosidase gene (beta-GAL-alpha) to chromosome 3 in man. *Cytogenet Cell Genet* 1978; **22**: 219.
16. Oshima A, Tsuji A, Nagao Y *et al*. Cloning, sequencing, and expression of cDNA for human beta-galactosidase. *Biochem Biophys Res Commun* 1988; **157**: 238.
17. Nishimoto J, Nanba E, Inui K *et al*. GM₁-gangliosidosis (genetic beta-galactosidase deficiency): identification of four mutations in different clinical phenotypes among Japanese patients. *Am J Hum Genet* 1991; **49**: 566.
18. Yoshida K, Oshima A, Shimmoto M *et al*. Human beta-galactosidase gene mutations in GM₁-gangliosidosis: a common mutation among Japanese adult/chronic cases. *Am J Hum Genet* 1991; **49**: 435.
19. Oshima A, Yoshida K, Shimmoto M *et al*. Human beta-galactosidase gene mutations in Morquio B disease. *Am J Hum Genet* 1991; **49**: 1091.
20. Suzuki Y, Oshima A. A β -galactosidase gene mutation identified in both Morquio B disease and infantile GM₁-gangliosidosis. *Hum Genet* 1993; **91**: 407.
21. Stone DL, Sidransky E. Hydrops fetalis: lysosomal storage disorders in extremis. *Adv Pediatr* 1999; **46**: 409.
22. Tasso MJ, Martinez-Gutierrez A, Carrascosa C *et al*. GM₁-gangliosidosis presenting as nonimmune hydrops fetalis: a case report. *J Perinat Med* 1996; **24**: 445.
23. Emery JM, Green WR, Wyllie RG, Howell RR. GM₁ gangliosidosis. *Arch Ophthalmol* 1971; **85**: 177.
24. Barbarik A, Benson PF, Fenson AH, Barrie H. Corneal clouding in GM₁ generalized gangliosidosis. *Br J Ophthalmol* 1976; **60**: 565.
25. Hooft C, Senesael L, Delbeke MJ *et al*. The GM₁-gangliosidosis (Landing disease). *Eur Neurol* 1969; **2**: 225.
26. Hubain P, Adam E, Dewelle A *et al*. Etude d'une observation de gangliosidose à GM₁. *Helv Paediatr Acta* 1969; **24**: 337.
27. Beratis NG, Varvarigou-Frimas A, Beratis S, Sklower SL. Angiokeratoma corporis diffusum in GM₁ gangliosidosis, type 1. *Clin Genet* 1989; **36**: 59.
28. Rosenberg H, Frewen TC, Li MD *et al*. Cardiac involvement in diseases characterized by β -galactosidase deficiency. *J Pediatr* 1985; **106**: 78.
29. Kohlschütter A, Sieg K, Schuyt FJ *et al*. Infantile cardiomyopathy and neuromyopathy with beta-galactosidase deficiency. *Eur J Pediatr* 1982; **139**: 75.
30. Benson PF, Babarik A, Brown SP, Mann TP. GM1-generalized gangliosidosis variant with cardiomegaly. *Postgrad Med J* 1976; **52**: 159.
31. Fricker H, O'Brien JS, Vassella F *et al*. Generalized gangliosidosis: acid β -gangliosidase deficiency with early onset, rapid mental deterioration and minimal bone dysplasia. *J Neurol* 1976; **12**: 329.
32. Spranger JW, Langer LEO, Wiedemann HR. GM₁ gangliosidosis. In: Spranger JW, Langer LEO, Wiedemann HR (eds). *Bone Dysplasias: An Atlas of Constitutional Disorders of Skeletal Development*. Philadelphia: WB Saunders Co, 1974: 171.
33. Suzuki K, Suzuki K, Chen GC. Morphological, histochemical and biochemical studies on a case of systemic late infantile lipidosis (generalized gangliosidosis). *J Neuropathol Exp Neurol* 1968; **27**: 15.
34. Curless RG. Computed tomography of GM₁ gangliosidosis. *J Pediatr* 1984; **105**: 964.
35. Kobayashi O, Takashima S. Thalamic hyperdensity on CT in infantile GM₁-gangliosidosis. *Brain Dev* 1994; **16**: 472.
36. Harden A, Martinovic Z, Pampiglione G. Neurophysiological studies in GM₁-gangliosidosis. *Ital J Neurol Sci* 1982; **3**: 201.
37. Pampiglione G, Harden A. Neurophysiological investigations in GM₁ and GM₂-gangliosidosis. *Neuropediatrics* 1984; **15**(Suppl.): 74.
38. Gonatas NK, Gonatas J. Ultrastructural and biochemical observations on a case of systemic late infantile lipidosis and its relationship to Tay-Sachs disease and gargoylism. *J Neuropathol Exp Neurol* 1965; **24**: 318.
39. Severi F, Magrini U, Tettamanti G *et al*. Infantile GM₁ gangliosidosis. Histochemical, ultrastructural and biochemical studies. *Helv Paediatr Acta* 1971; **26**: 192.
40. O'Brien JS, Bennett J, Veath ML, Paa D. Lysosomal storage disorders: diagnosis by ultrastructural examination of skin biopsies. *Arch Neurol* 1975; **32**: 592.
41. Suzuki K. Cerebral GM₁ gangliosidosis: Chemical pathology of visceral organs. *Science* 1968; **159**: 1471.
42. Wolfe LS, Calahan J, Fawcett JS *et al*. GM₁ gangliosidosis without chondrodystrophy or visceromegaly. *Neurology* 1970; **20**: 23.
43. Singer HS, Schafer IA. Clinical and enzymatic variations in GM₁ generalized gangliosidosis. *Am J Hum Genet* 1972; **24**: 454.
44. Patton VM, Dekaban AS. GM₁ gangliosidosis and juvenile cerebral lipidosis. *Arch Neurol* 1971; **24**: 529.
45. Generoso G, Gascon MD, Ozand PT, Erwin RE. GM₁ gangliosidosis type 2 in two siblings. *J Child Neurol* 1992; **7**: 41.
46. Owman T, Sjöblad S, Gohlin J. Radiographic skeletal changes in juvenile GM₁ gangliosidosis. *Fortschr Röntgenstr* 1980; **132**: 692.
47. Derry DM, Fawcett JS, Andermann F, Wolfe LS. Late infantile systemic lipidosis. Major monosialogangliosidosis. Delineation of two types. *Neurology* 1968; **18**: 340.
48. Takamoto K, Beppu H, Hirose K, Uono M. Juvenile β -galactosidase deficiency – a case with mental deterioration,

- dystonic movement, pyramidal symptoms, dysostosis and cherry red spot. *Clin Neurol (Tokyo)* 1980; **20**: 339.
49. Weissbluth M, Esterly NB, Caro WA. Report of an infant with GM₁ type I and extensive and unusual mongolian spots. *Br J Dermatol* 1981; **104**: 195.
 50. Selsor LC, Leshner JJJ. Hyperpigmented macules and patches in a patient with GM₁ type 1 gangliosidosis. *J Am Acad Dermatol* 1990; **20**: 878.
 51. Esterly NB, Weissbluth M, Caro WA. Mongolian spots and GM₁ type 1 gangliosidosis. *J Am Acad Dermatol* 1990; **22**: 320.
 52. Stevenson RE, Taylor HA, Parks S. β -Galactosidase deficiency: prolonged survival in three patients following early central nervous deterioration. *Clin Genet* 1978; **13**: 305.
 53. Wenger DA, Sattler M, Mueller OT *et al.* Adult GM₁ gangliosidosis: clinical and biochemical studies on two patients and comparison to other patients called variant or adult GM₁ gangliosidosis. *Clin Genet* 1980; **17**: 323.
 54. Yoshida K, Oshima A, Sakuraba H *et al.* GM₁-gangliosidosis in adults: clinical and molecular analysis of 16 Japanese patients. *Ann Neurol* 1992; **31**: 328.
 55. Goldman JE, Katz D, Rapin I *et al.* Chronic GM₁ gangliosidosis in presenting as dystonia: I. Clinical and pathological features. *Ann Neurol* 1981; **9**: 465.
 56. Kobayashi T, Suzuki K. Chronic GM₁ gangliosidosis in presenting as dystonia; II. Biochemical studies. *Ann Neurol* 1981; **9**: 476.
 57. Yamamoto A, Adachi S, Kawamura S *et al.* Localised β -galactosidase deficiency. Occurrence in cerebellar ataxia with myoclonus epilepsy and macular cherry-red spot – a new variant of GM₁-gangliosidosis? *Arch Intern Med* 1974; **134**: 627.
 58. Groebe H, Krins M, Schmidberger H *et al.* Morquio syndrome (mucopolysaccharidoses IV B) associated with β -galactosidase deficiency. Report of two cases. *Am J Hum Genet* 1980; **32**: 258.
 59. van Gemund JJ, Giesberts MA, Eerdmans RF *et al.* Morquio-B disease, spondyloepiphyseal dysplasia associated with acid β -galactosidase deficiency. Report of three cases in one family. *Hum Genet* 1983; **64**: 50.
 60. Giugliani R, Jackson M, Skinner SJ *et al.* Progressive mental regression in siblings with Morquio disease Type B (mucopolysaccharidoses IV B). *Clin Genet* 1987; **32**: 313.
 61. Farrell DF, Ochs U. GM₁: Phenotypic variation in a single family. *Ann Neurol* 1981; **9**: 225.
 62. Singer HS, Schafer IA. White cell β -galactosidase activity. *N Engl J Med* 1970; **282**: 571.
 63. Lowden JA, Cutz E, Conen PE *et al.* Prenatal diagnosis of GM₁ gangliosidosis. *N Engl J Med* 1973; **288**: 255.
 64. Kaback MM, Sloan HR, Sonneborn M *et al.* GM₁ gangliosidosis type I *in utero*: detection and fetal manifestations. *J Pediatr* 1973; **82**: 1037.
 65. Booth CW, Gerbie AB, Nadler HL. Intrauterine diagnosis of GM₁ gangliosidosis, type 2. *Pediatrics* 1973; **52**: 521.
 66. Warner TG, Robertson AD, Mock AK *et al.* Prenatal diagnosis of GM₁ gangliosidosis by detection of galactosyl-oligosaccharides in amniotic fluid with high performance liquid chromatography. *Am J Hum Genet* 1983; **35**: 1034.
 67. Kleijer WJ, Van der Veer E, Niermeijer MF. Rapid prenatal diagnosis of GM₁-gangliosidosis using microchemical methods. *Hum Genet* 1976; **33**: 299.
 68. Brezina PR, Benner A, Rechitsky S *et al.* Single-gene testing combined with single nucleotide polymorphism microarray preimplantation genetic diagnosis for aneuploidy: a novel approach in optimizing pregnancy outcome. *Fertil Steril* 2011; **95**: 1786.
 69. Norden AGW, O'Brien JS. Ganglioside GM₁ β -galactosidase: studies in human liver and brain. *Arch Biochem Biophys* 1973; **159**: 383.
 70. Frost RG, Holmes EW, Norden AGW, O'Brien JS. Characterization of purified human liver acid β -galactosidases A₂ and A₃. *Biochem J* 1978; **175**: 181.
 71. D'Azzo A, Hoogeveen A, Reuser ADJ *et al.* Molecular defect in combined β -galactosidase and neuroaminidase deficiency. *Proc Natl Acad Sci USA* 1982; **79**: 4535.
 72. Shows T, Scrafford-Wolff LR, Brown JA, Meisler MH. GM₁ gangliosidosis: chromosome 3 assignment of the β -galactosidase gene. *Somatic Cell Genet* 1979; **5**: 147.
 73. Takano T, Yamanouchi Y. Assignment of human β -galactosidase-A gene to 3p21.33 by fluorescence *in situ* hybridization. *Hum Genet* 1993; **92**: 403.
 74. Hoogeveen AT, Verheijen FW, Galjaard H. The relationship between human lysosomal β -galactosidase and its protective protein. *J Biol Chem* 1983; **258**: 12 143.
 75. O'Brien JS. Molecular genetics of GM₁ β -gangliosidosis. *Clin Genet* 1975; **8**: 303.
 76. Macbrinn MC, Okada S, Ho MW *et al.* Generalized gangliosidosis: impaired cleavage of galactose from a mucopolysaccharide and a glycoprotein. *Science* 1969; **163**: 946.
 77. Singer HS, Nankervis GA, Schafer IA. Leukocyte beta-galactosidase activity in the diagnosis of generalized GM₁ gangliosidosis. *Pediatrics* 1972; **49**: 352.
 78. Sloan HR, Uhlendorf W, Jacobson CB, Frederickson DS. β -Galactosidase in tissue culture derived from human skin and bone marrow: enzyme defect in GM₁ gangliosidosis. *Pediatr Res* 1969; **3**: 532.
 79. Yutaka T, Okada S, Kato T, Yabuuchi H. Impaired degradation of keratan sulfate in GM₁-gangliosidosis. *Clin Chim Acta* 1982; **125**: 233.
 80. Beck M, Petersen EM, Spranger J, Beighton P. Morquio's disease type B (β -galactosidase deficiency) in three siblings. *S Afr Med J* 1987; **72**: 704.
 81. Pinsky L, Powell E, Callahan J. GM₁-gangliosidosis types 1 and 2: enzymatic differences in cultured fibroblasts. *Nature* 1970; **228**: 1093.
 82. Wolfe LS, Senior RG, Ng Y, Kin NMK. The structures of oligosaccharides accumulating in the liver of GM₁ gangliosidosis, type 1. *J Biol Chem* 1974; **249**: 1828.
 83. Strecker G, Montreuil JG. Glycoproteins et glycoproteinoses. *Biochimie* 1979; **61**: 1199.
 84. Yamashita K, Ohkura T, Okada S *et al.* Urinary oligosaccharides of GM₁ gangliosidosis. Different excretion patterns in the urine of type 1 and type 2 subgroups. *J Biol Chem* 1981; **256**: 45 789.
 85. Ohkura T, Yamashita K, Kobata Q. Urinary oligosaccharides of GM₁-gangliosidosis. Structure of oligosaccharides excreted in the urine of type 1 but not in the urine of type 2 patients. *J Biol Chem* 1981; **256**: 8485.

86. Warner TG, Robertson AD, O'Brien JS. Diagnosis of GM₁ gangliosidosis based on defect in the urinary oligosaccharides with high performance liquid chromatography. *Clin Chim Acta* 1983; **127**: 313.
87. Holmes Z, O'Brien JS. Separation of glycoprotein derived oligosaccharides by thin-layer chromatography. *Anal Biochem* 1979; **93**: 167.
88. Ledeen R, Wu G. GM₁ in the nuclear envelope regulates nuclear calcium through association with a nuclear sodium-calcium exchanger. *J Neurochem* 2007; **103**(Suppl. 1): 126.
89. Sano R, Annunziata I, Patterson A *et al*. GM₁-ganglioside accumulation at the mitochondria-associated ER membranes links ER stress to Ca(2+)-dependent mitochondrial apoptosis. *Mol Cell* 2009; **36**: 500.
90. Oshima A, Yoshida K, Ishizaki A *et al*. GM₁-gangliosidosis: tandem duplication within exon 3 of β -galactosidase gene in an infantile patient. *Clin Genet* 1992; **41**: 235.
91. Boustany R-M, Qian W-H, Suzuki K. Mutations in acid β -galactosidase cause GM₁-gangliosidosis in American patients. *Am J Hum Genet* 1993; **53**: 881.
92. Mosna G, Fattore S, Tubiello G *et al*. A homozygous missense arginine to histidine substitution at position 482 of the β -galactosidase in an Italian infantile GM₁-gangliosidosis patient. *Hum Genet* 1992; **90**: 247.
93. Yang CF, Wu JY, Tsai FJ. Three novel beta-galactosidase gene mutations in Han Chinese patients with GM1 gangliosidosis are correlated with disease severity. *J Biomed Sci* 2010; **17**: 79.
94. Brunetti-Pierri N, Scaglia F. GM1 gangliosidosis: review of clinical, molecular, and therapeutic aspects. *Mol Genet Metab* 2008; **94**: 391.
95. Hofer D, Paul K, Fantur K *et al*. Phenotype determining alleles in GM1 gangliosidosis patients bearing novel GLB1 mutations. *Clin Genet* 2010; **78**: 236.
96. Baker HJ, Lindsay JR. Feline GM₁-gangliosidosis. *Am J Pathol* 1974; **74**: 649.
97. Read DH, Harrington DD, Kenan TW, Hinsman EJ. Neuronal visceral GM₁ gangliosidosis in a dog with β -gangliosidase deficiency. *Science* 1976; **194**: 442.
98. Ushiyama M, Hanyu N, Ikeda S, Yanagisawa N. A case of type III (adult) GM₁-gangliosidosis that improved markedly with trihexyphenidyl. *Clin Neurol (Tokyo)* 1986; **26**: 221.
99. Baek RC, Broekman ML, Leroy SG *et al*. AAV-mediated gene delivery in adult GM1-gangliosidosis mice corrects lysosomal storage in CNS and improves survival. *PLoS One* 2010; **5**: e13468.
100. Kasperzyk JL, El-Abbadi MM, Hauser EC *et al*. N-utyldeoxygalactonojirimycin reduces neonatal brain ganglioside content in a mouse model of GM₁ gangliosidosis. *J Neurochem* 2004; **89**: 645.
101. Matsudo J, Suzuki O, Oshima A *et al*. Chemical chaperone therapy for brain pathology in GM₁-gangliosidosis. *Proc Nat Acad Sci USA* 2003; **100**: 15 912.
102. Suzuki Y. Chemical chaperone therapy for GM1-gangliosidosis. *Cell Mol Life Sci* 2008; **65**: 351.
103. Fantur K, Hofer D, Schitter G *et al*. DLHex-DGJ, a novel derivative of 1-deoxygalactonojirimycin with pharmacological chaperone activity in human G(M1)-gangliosidosis fibroblasts. *Mol Genet Metab* 2010; **100**: 262.
104. Schitter G, Steiner AJ, Pototschnig *et al*. Fluorous iminoalditols: a new family of glycosidase inhibitors and pharmacological chaperones. *ChemBiochem* 2010; **11**: 2026.
105. Schitter G, Scheucher E, Steiner AJ *et al*. Synthesis of lipophilic 1-deoxygalactonojirimycin derivatives as D-galactosidase inhibitors. *Beilstein J Org Chem* 2010; **6**: 21.

Tay-Sachs disease/hexosaminidase A deficiency

Introduction	678	Treatment	683
Clinical abnormalities	678	References	683
Genetics and pathogenesis	681		

MAJOR PHENOTYPIC EXPRESSION

Infantile cerebral and retinal degeneration with cherry red macular spots, hyperacusis, macrocephaly, storage of GM₂ ganglioside in the brain, and deficiency of hexosaminidase A.

INTRODUCTION

Tay-Sachs disease has been described as the prototype of the lysosomal storage disorders [1]. It represents a paradigm for the success of research in biochemical genetics not only in providing precise molecular understanding of the nature of disease but also in the practical community-based control of a genetic disease.

The disease was first described by Tay [2] in 1881 in an infant in whom a cherry macular spot was associated with delayed development. Sachs [3] defined the clinical entity, which he called a familial amaurotic idiocy [4]. The enzymatic defect was discovered in 1969 by Okada and O'Brien [5]. The deficiency in hexosaminidase A results in a failure to cleave the terminal N-acetylgalactosamine (GalNAc) from the GM₂ ganglioside (Figure 90.1). The development of methodology for the rapid, relatively easy quantification of the A isozyme has permitted accurate identification of heterozygous carriers of the gene and prenatal diagnosis, permitting a public health approach to human genetics and the virtual prevention of the birth of affected children in the population at highest risk [6, 7].

The various disorders of ganglioside GM₂ storage are summarized in Table 90.1. All are progressive cerebral degenerative diseases. The cherry red macular spot is a prominent feature in all of the early infantile presentations, all of which are fatal in infancy. All of these diseases are autosomal recessive. Neuronal lipidosis is a common histologic feature and results from the storage of ganglioside. Deficiency of lysosomal hydrolase activity provides in each a molecular explanation for the disease

in which storage of GM₂ ganglioside results from failure to cleave its terminal GalNAc. There are three types of GM₂ gangliosidosis. Sandhoff disease (Chapter 91) has been referred to as the O variant to indicate that neither hexosaminidase A nor hexosaminidase B is active. In this classification Tay-Sachs disease was termed the B variant, since only the B isozyme is active; in what was called the AB variant both enzymes are active and the defect is in the GM₂ activator (Chapter 92).

Hexosaminidase A is a heterodimer containing the α and β subunits. Hexosaminidase B is composed of two β subunits. Cultured cells of patients with Tay-Sachs disease lack the α chain. The genes for the α and β chains have been cloned and the locus for the α chain and for Tay-Sachs disease is on chromosome 15q23 [8]. The gene is common in Ashkenazi Jews. A considerable number and variety of mutations have been described, most in patients with the classic infantile phenotype [9, 10]. The most frequent mutation in the Ashkenazi Jewish population is a four-nucleotide insertion in exon 11, which introduces a frame shift and a downstream premature termination signal that results in a deficiency of mRNA.

CLINICAL ABNORMALITIES

Patients with Tay-Sachs disease appear normal at birth, although storage of GM₂ ganglioside has been demonstrated even in the fetus [11]. Infants continue to appear alert and healthy until about six months of age. The onset of clinical disease may be between birth and

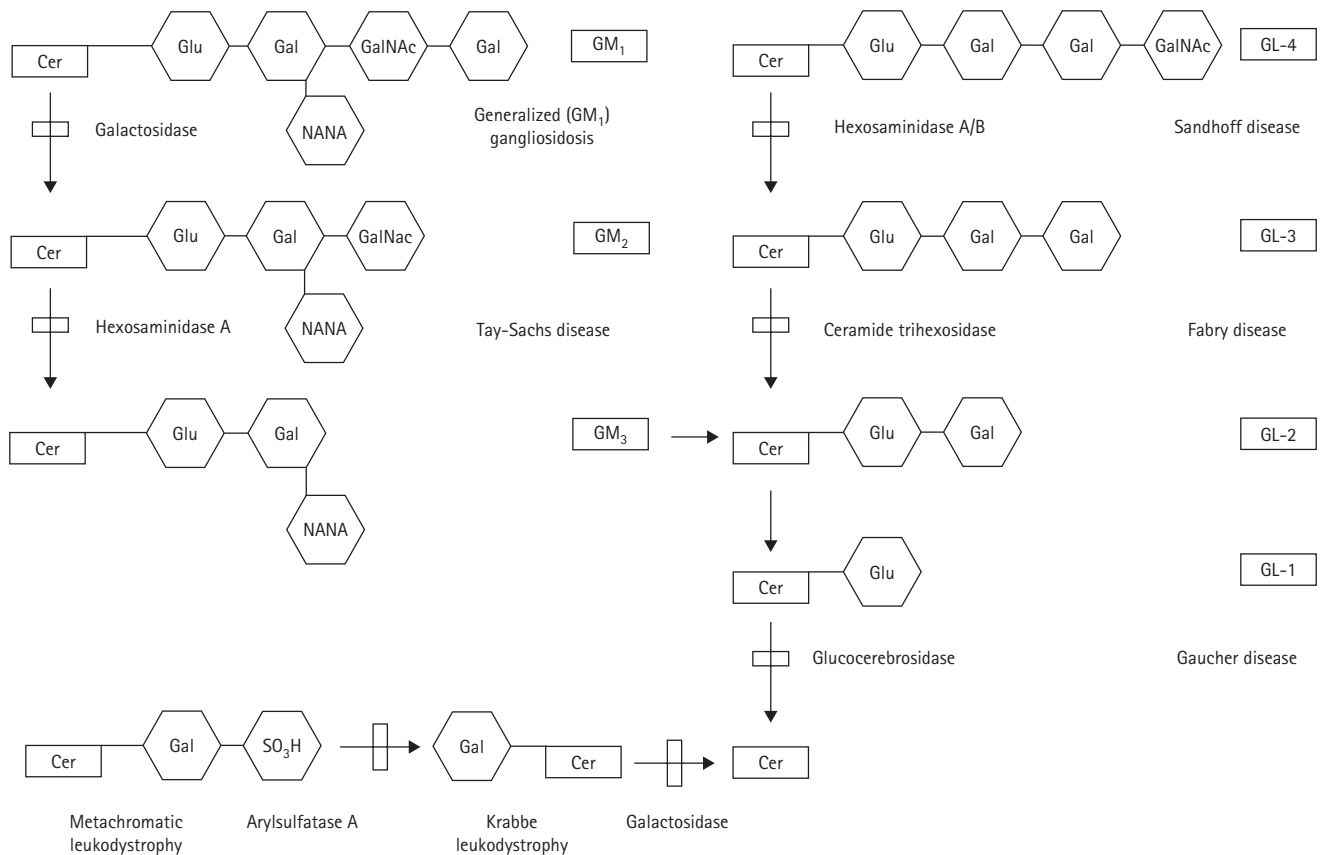


Figure 90.1 Metabolic pathways of glycosphingolipid metabolism. The site of the defect in a number of conditions is illustrated. That of Tay-Sachs disease is in hexosaminidase A, which catalyzes the removal of N-acetyl-galactosamine from the GM₂ lipid to produce GM₃. Cer, ceramide; Glu, glucose; Gal, galactose; NANA, N-acetyl-neuraminic acid; and GalNAc, N-acetylgalactosamine.

Table 90.1 GM₂ ganglioside storage diseases

Disorder	Age at onset	Age at death (years)	Enzyme defect	Carrier detection	Prenatal diagnosis
Tay-Sachs disease	3–6 months	2–4	Hexosaminidase A	+	Established
Sandhoff disease	3–6 months	2–4	Hexosaminidase A and B	+	Established
AB variant	3–6 months		Activator	+	Possible
Juvenile GM ₂ gangliosidosis	2–6 years	5–15	Hexosaminidase A	+	Possible
Adult GM ₂ gangliosidosis	2 years–adulthood	Variable	Hexosaminidase A	+	Possible

ten months of age [12]. The earliest clinical manifestation may be an exaggerated startle response to sound, in which the arms and legs extend. This usually is present by one month of age, but it may not be appreciated early, since it can be seen in some normal babies, usually disappearing in about four months. In contrast, in the baby with Tay-Sachs disease this hyperacusis becomes more prominent. It is brought on even by very gentle sound stimuli. It may be accompanied by clonus.

Parents may notice motor weakness as the first clinical sign. By eight months of age, the baby may look sleepy or less alert. The infant may begin to sit less well or to begin to lose head control. Physical examination at this stage reveals hypotonia. This is progressive. By one year of age,

few of these patients can sit without support. The usual developmental milestones are lost or never attained. There may be nystagmus and a fixed, staring or roving gaze. Examination of the fundus reveals the typical cherry red spot in the macula (Figure 90.2). This is usually present as early as two months of age and has been demonstrated by retinal photography as early as the first days of life. In looking for this it is important to remember that the white degeneration of the macula is larger and more impressive than the red foveal spot in the middle. Together they look very much like a fried egg. Lipid storage in the ganglion cells obscures the choroidal vessels behind. In the fovea, where ganglion cells are few in number, vascularity of the choroid is seen as the red spot. With time the spot may



Figure 90.2 The cherry red spot. Photograph of the fundus of an infant with Tay-Sachs disease.



Figure 90.4 The lower extremities of the same patient were flaccid.



Figure 90.3 AS: A two-year-old patient with Tay-Sachs disease. He was blind and decerebrate at this time. Hexosaminidase A activity was absent.



Figure 90.5 MAS: A two-year-old boy with Tay-Sachs disease. He was hypotonic but had increased deep tendon reflexes and positive Babinski responses. He had hyperacusis and was blind.

become darker or brownish in color. Opaque white streaks may develop along the vessels.

Cerebral and macular degeneration is rapidly progressive. The infant becomes blind, rigid, and decerebrate by 12–18 months ([Figure 90.3](#)), and usually must be fed by tube because swallowing is ineffective. The extremities may be flaccid ([Figure 90.4](#)), but muscle tone is usually increased, and there is hyperreflexia ([Figures 90.5](#) and [90.6](#)) and opisthotonos. Convulsions and myoclonic jerks are common. Seizures almost invariably occur after one year of age, but they are not difficult to control with anticonvulsant medication. Electroencephalograph (EEG) abnormalities are relatively mild but become progressive

after the first year [13]. The electroretinogram is normal but visual evoked potentials disappear.

Patients often have a doll-like facial appearance ([Figure 90.3](#)) with clear, translucent skin, long eyelashes, fine hair and delicate pink coloring. After about 15 months the head size usually enlarges. By this time there is decerebrate posturing, difficulty with swallowing and secretions, and vegetative unresponsiveness. The brain weight at the time of death may be 50 percent heavier than normal. This is a consequence of glial proliferation and of lipid storage. There is no hepatosplenomegaly or other peripheral evidence of storage disease.

Death usually results from aspiration and pneumonia;



Figure 90.6 FHS: A 16-month-old girl with Tay-Sachs disease. She was hypotonic but hyperreflexic and had bilateral ankle clonus and positive Babinski responses. Pupils reacted poorly to light, and she had bilateral cherry red spots.

usually by two to four years of age. Pathologic changes are restricted to the nervous system, where the neurons are swollen, or 'ballooned,' displacing the nucleus toward the periphery [14]. This picture may be seen in neurons of the autonomic system and rectal mucosa as well as in the cerebral cortex. 'Meganeurites' have been described among cortical neurons [15]. Electron microscopy of the neuron reveals lamellar membranous cytoplasmic bodies [16]. These inclusions are round concentric layers of accumulated ganglioside cholesterol and phospholipid in lysosomes. Pathologic changes can be demonstrated by electron microscopic study of biopsied skin [17, 18]. As axonal degeneration proceeds in the brain, there is secondary demyelination and cortical gliosis. Membranous cytoplasmic bodies have been found in fetal brain as early as 12 weeks [19].

A certain amount of genetic heterogeneity has been established among GM₂ gangliosidoses [20–22]. The first to be appreciated was Sandhoff disease (Chapter 91), in which there is deficiency of both hexosaminidases A and B and a phenotype indistinguishable from that of Tay-Sachs disease. This is also true of GM₂ activator deficiency (Chapter 92).

A more indolent phenotype referred to as juvenile GM₂ gangliosidosis has an onset at about two years of age with ataxia and incoordination. Speech is lost and deterioration is progressive to spasticity and decerebrate rigidity. Activity of hexosaminidase A is deficient but not to the degree seen in Tay-Sachs disease [20, 23]. A less severe

form of GM₂ gangliosidosis has been referred to as adult or chronic GM₂ gangliosidosis [24]. This and the juvenile phenotype represent parts of a spectrum of genetically determined variants, ranging from the classic infantile Tay-Sachs phenotype to adult disease that progresses so slowly intellect is hardly affected. The advent of molecular biology and the very extensive documentation of mutation make most of these clinical classifications obsolete.

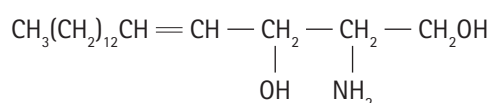
Prominent features in variant patients are distal or proximal muscle atrophy, pes cavus or foot drop, as well as spasticity, dystonic movements, dysarthria, and ataxia. The overall picture may be reminiscent of spinocerebellar degeneration [24, 25]. Some patients [26] have been clinically normal when first diagnosed on the basis of low enzyme activity, but later have developed difficulties of speech and gait. Others have been studied [27, 28] in whom clinical manifestations have not been observed. Psychotic disease has been common in adult onset GM₂ gangliosidosis [29, 30]. Response to antipsychotic medication has been poor. Some patients have been observed with problems of supranuclear gaze [31], raising the differential diagnosis of Niemann-Pick type C disease (Chapter 95).

An assay has been developed [23] in which fibroblasts of the various phenotypes have had activities of hexosaminidase A that correlated well with the clinical picture. In this assay patients with Tay-Sachs disease displayed 0.1 percent of control activity, the late-infantile or so-called juvenile 0.5 percent, and the adult GM₂ gangliosidosis 2–4 percent. The clinically asymptomatic individuals with low hexosaminidase activity had 11–20 percent of control activity.

GENETICS AND PATHOGENESIS

Tay-Sachs disease is transmitted as an autosomal recessive disease. The gene frequency in Ashkenazi Jews has been calculated to be approximately one in 30 [6]. This would predict an annual incidence of one in 4000 births with Tay-Sachs disease among parents from this population. These frequencies were so high that it became practical to undertake programs of prevention through heterozygote detection. Gene frequency in non-Jews has been calculated to be one in 300 [6, 32]. The disease is also common in some isolates in Switzerland [33] and in French descendants in Eastern Quebec and Southern Louisiana [34].

The GM₂ ganglioside stored in Tay-Sachs disease is an acidic glycosphingolipid with a terminal hexosamine, GalNAc (Figure 90.1) [35–38]. The ganglioside, which is normally present in very small amounts, is increased 100 to 1000 times in Tay-Sachs disease. The sphingolipids all contain the long-chain base sphingosine, which has the following structure:



This compound is acylated with long-chain fatty acids on the amino group on carbon 2 to form ceramide, which makes up the base unit of all of the sphingolipids. In the gangliosides, as well as in the cerebroside and glycolipids, a sugar is linked glucosidically to carbon 1. In the parent ganglioside, GM₁, the glycolipid that accumulates in generalized gangliosidosis, ceramide is linked successively to glucose; to galactose, to which N-acetylneuraminic acid is attached; to N-acetylgalactosamine; and to galactose. This terminal galactose is cleaved by a β -galactosidase, which is defective in generalized GM₁ gangliosidosis, to yield the Tay-Sachs lipid, GM₂ [36]. This is normally converted to GM₃ by cleavage of the terminal GalNAc. The amounts of GM₂ storage in the brain in variant patients tend to be less than in Tay-Sachs disease [39, 40].

The defect in hexosaminidase A represents a failure to hydrolyze this terminal aminosugar from the GM₂ ganglioside. It was first demonstrated by Okada and O'Brien [5] by starch gel electrophoresis which separated hexosaminidase activity into two components, designated A and B, the former (A) of which was absent in patients with Tay-Sachs disease. The A enzyme is more heat-labile and negatively charged than the B isozyme [38]. The diagnosis is made by measuring total and heat-stable hexosaminidase activity in serum, using artificial methylumbelliferyl N-acetylgalactosamine or N-acetylglucosamine substrate whose product of cleavage is the fluorogenic 4-methylumbelliferone [41]. The heat-labile enzyme is hexosaminidase A, and its activity is represented by the difference in activity before and after denaturation. The enzyme can also be measured in freshly isolated leukocytes, tears and cultured fibroblasts or amniotic fluid cells [42]. In Tay-Sachs disease the activity of hexosaminidase A is virtually zero [5, 21, 43]. Assays have also employed [20, 21] the natural substrate ganglioside GM₂.

Cross-reacting material (CRM) was demonstrated using antibodies prepared against human placental hexosaminidase A and the α -subunit of kidney and liver extracts of some patients with Tay-Sachs disease [44], but most are CRM-negative. The gene for the α subunit of hexosaminidase A contains 14 exons over 35 kb and 5' regulatory elements (TATA) and 3' untranslated areas [45]. Very many mutations have been documented [6, 10, 46–50] spanning all 14 exons. Twenty-one of these occur at CpG dinucleotide sites, which are known to be mutagenic hot spots and which account for more than one-third of human polymorphisms and disease mutations [47, 48]. Even within the classic Tay-Sachs infantile phenotype, almost 100 different mutations have been reported [6, 9, 10]. Deletions in sections, frameshifts, and stop codons are found in this phenotype.

Two mutations account for 93 percent of the mutant alleles in the Ashkenazi Jewish population of North America (Table 90.2) [9, 46]. In addition to the 4 bp insertion, 1278ins4, the other common deletion in this population is a splice site inversion in intron 12. A G-to-C change in the first nucleotide of the intron leads to several

Table 90.2 Mutations in the hexosaminidase A gene in Tay-Sachs disease

Mutation	Population	Frequency
+TATC 1278ins4	Ashkenazi Jewish	80%
+IVS 12 (G→C)	Ashkenazi Jewish	15%
G269S	Ashkenazi Jewish	Most late onset
910 del TTC	Moroccan Jewish	Most of that population
–IVS 5-1G→T	Japanese	Most of that population
+IVS 7 (G→A)	French Canadian	Rare
+IVS 9+1(G→A)	Celtic, French, Pennsylvania Dutch	Rare
Δ Phe304 or 305	Moroccan, Jewish, Irish, French	Rare
–IVS4 (G→T)	Armenian, Black	Rare
C deletion 1510	Italian	Rare
A→G, exon 1	American Black	Rare
G436 deletion (exon 4)	American Black	Rare
C→T 409 (exon 3)	American non-Jewish, Caucasian	Rare

Assembled from data reported by Kaback *et al.* [6].

abnormally spliced mRNAs. The other common mutation is the French Canadian mutation, a 7.6 kb deletion in exon 1 and flanking sequences in a population in which the frequency of the disease has been similar to that of the Ashkenazi Jewish population [51].

All of the other mutations are rare and have been found generally in compounds except in consanguineous families. The Δ Phe 304 or 305 represents deletion of one of two adjacent phenylalanine moieties [48]. The mutation found in two unrelated black American families [49] was an interesting one in which a G-to-T transversion in the invariant AG of the acceptor splice site of intron 4 interfered with splicing. An A-to-G transition in exon 1 found in a black American family changed the initiating methionine to a valine. Of the last two mutations in Table 90.2, C to T409 creates a termination codon, and deletion of G436 frameshift leads to a termination codon. Some interesting mutations interfere with the assembly or processing of a synthesized α subunit. R504C and R504H are secreted, and not retained in early compartments but fail to associate with β subunits to form the enzyme [48, 52].

The mutations associated with the later-onset phenotypes of GM₂ gangliosidosis have generally been single-base substitutions, leading to a single amino acid change [6]. Many have been found in compounds. In one [53], a mutation, G570A, led to alternate splicing in which a certain amount of normal mRNA was made, while in

Table 90.3 Prevention of Tay-Sachs disease (1971–92), showing >90 percent reduction in the disease in Jewish population (1970–93)

Group	Number
Total screened	9.53 × 10 ⁶ (seven countries)
Carriers identified	36,418
Couples at risk	1056
Pregnancies monitored	2415 ^a
Affected fetuses	469
Aborted	451
Normal offspring born	1881
Births per year with Tay-Sachs:	
Prior to 1969	100 (US and Canada) (80% Jewish)
1980	13 (80% non-Jewish)
1985–92	3–10 (85% non-Jewish)

^aPrior offspring as well as heterozygote screening (1969–1992).

most of the mRNA exon 5 was missing. The adult-onset phenotype in Jewish populations results in a glycine 269 to serine change [54].

Heterozygous carriers of the gene have intermediate activities of hexosaminidase A in their serum or plasma [41]. These values average 65 percent of those of normal. Screening for heterozygosity should be done prior to the development of pregnancy, since this may cause a false positive result in the serum assay. The issue can be resolved by assay of leukocytes.

The heat denaturation assay has been automated and employed in mass screening for heterozygotes throughout the world [55–57]. Such programs have shown that the disease can be virtually eliminated in those at highest risk [6]. Between 1971 and 1992, almost a million people were screened for heterozygosity in 17 countries (Table 90.3). Of these, over 36,000 carriers were detected and 1000 couples at risk were identified because both were carriers. Considering couples identified by screening and also those identified because of prior offspring, 2416 pregnancies were monitored and 469 affected fetuses were found, of which all but 18 were aborted. More important than the prevention of all these patients with Tay-Sachs disease, almost 2000 normal offspring were born to these couples at risk. When this program began, 50–100 infants with Tay-Sachs disease were born annually in the United States and Canada, 80 percent of whom were Jewish. In the past 20 years, the incidence has varied between three and ten annually, of whom 85 percent were non-Jewish. This represents a 90 percent reduction in the incidence of Tay-Sachs disease in the Jewish population of the two countries.

Tay-Sachs disease can be detected prenatally [11].

The enzyme is reliably assayed in cultured amniotic fluid cells or chorionic villus material [6, 58]. Tay-Sachs is the metabolic disease most frequently diagnosed prenatally [6]. Early pitfalls or misdiagnoses have been eliminated by regular use of cultured amniotic fluid cells for assay, prior establishment that both parents are heterozygotes [59], and the use of ultrasonography to rule out the presence of twinning. Even so, there have been a few misdiagnoses, all attributable to such laboratory errors as mix-ups of samples.

Testing of potential heterozygotes by enzyme assay with synthetic substrates has uncovered the existence of pseudodeficiency genes that can result in a healthy person who has no hexosaminidase A activity in this assay. Approximately 35 percent of non-Jewish persons identified as heterozygotes by enzyme assay are pseudodeficiency carriers [60]. Many of the individuals have been identified to have a mutation substituting tryptophan 247 for arginine [61]. The existence of the pseudodeficiency allele makes it essential to do mutational analysis in couples at risk because both have been identified as carriers by enzyme analysis. There is no risk if one or both carry the pseudodeficiency allele. In families in which mutation has been identified, analysis for mutation can be employed for heterozygote detection and prenatal diagnosis [62].

TREATMENT

Specific treatment has not been developed. Skilled supportive care should be provided for the family and the patient should be made as comfortable as possible.

REFERENCES

1. Kaback MM, O'Brien JS. Tay-Sachs: prototype for prevention of genetic disease. *Hosp Pract* 1973; **8**: 107.
2. Tay W. Symmetrical changes in the region of the yellow spot in each eye of an infant. *Trans Ophthalmol Soc UK* 1881; **1**: 1155.
3. Sachs B. On arrested cerebral development with special reference to its pathology. *J Nerv Ment Dis* 1887; **14**: 541.
4. Sachs B. A family form of idiocy generally fatal associated with early blindness. *J Nerv Ment Dis* 1896; **21**: 475.
5. Okada S, O'Brien JS. Tay-Sachs disease: generalized absence of a b-d-N-acetylhexosaminidase component. *Science* 1969; **165**: 698.
6. Kaback M, Lim-Steele J, Dabholkar D *et al*. Tay-Sachs disease—carrier screening, prenatal diagnosis and the molecular era. An international perspective 1970 to 1993. *J Am Med Assoc* 1993; **270**: 2307.
7. Kaplan F. Tay-Sachs disease carrier screening: a model for prevention of genetic disease. *Genet Test* 1998; **2**: 271.
8. Gilbert F, Kucherlapati R, Creagan RP *et al*. Tay-Sachs and Sandhoff's diseases: the assignment of genes for hexosaminidases A and B to individual human chromosomes. *Proc Natl Acad Sci USA* 1975; **72**: 263.

9. Paw BH, Tieu PT, Kaback MM *et al.* Frequency of three Hex A mutant alleles among Jewish and non-Jewish carriers identified in a Tay-Sachs screening program. *Am J Hum Genet* 1990; **47**: 698.
10. Triggs-Raine BL, Feigenbaum ASJ, Natowicz M *et al.* Screening for carriers of Tay-Sachs disease among Ashkenazi Jews: a comparison of DNA-based and enzyme-based tests. *N Engl J Med* 1990; **323**: 6.
11. O'Brien JS, Okada S, Fillerup DL *et al.* Tay-Sachs disease: prenatal diagnosis. *Science* 1971; **172**: 61.
12. Volk BW (ed.). *Tay-Sachs Disease*. New York: Grune and Stratton, 1964.
13. Pampiglione G, Privett G, Harden A. Tay-Sachs disease: neurophysiological studies in 20 children. *Dev Med Child Neurol* 1974; **16**: 201.
14. Volk BS, Schneek L, Adachi M. Clinic pathology and biochemistry of Tay-Sachs disease. In: Vinken PJ, Bruyn GW (eds). *Textbook of Neurology*. Amsterdam: North-Holland, 1970; **10**: 385.
15. Purpura DP, Suzuki K. Distortion of neuronal geometry and formation of aberrant synapses in neuronal storage disease. *Brain Res* 1976; **116**: 1.
16. Terry RD, Weiss M. Studies in Tay-Sachs disease. II. Ultrastructure of the cerebrum. *J Neuropathol Exp Neurol (Berl)* 1973; **24**: 43.
17. Martin JJ, Jacobs K. Skin biopsy as a contribution to diagnosis in late infantile amaurotic idiocy with curvilinear bodies. *Eur Neurol* 1973; **10**: 281.
18. O'Brien JS, Bennett J, Veath ML, Paa D. Lysosomal storage disorders: diagnosis by ultrastructural examination of skin biopsy specimens. *Arch Neurol* 1975; **32**: 592.
19. Myrianthopoulos N, Aronson S. Reproductive fitness and selection. In: Aronson S, Volk B (eds). *Tay-Sachs Disease and Inborn Errors of Sphingolipid Metabolism*. New York: Pergamon Press, 1967.
20. O'Brien JS, Norden AGW, Miller AL *et al.* Ganglioside GM₂ N-acetyl-beta-D-galactosaminidase and asialo GM₂ (GA₂) N-acetyl-beta-D-galactosaminidase: studies in human skin fibroblasts. *Clin Genet* 1977; **11**: 171.
21. O'Brien JS, Tennant LL, Veath ML *et al.* Characterization of unusual hexosaminidase A-deficient human mutants. *Am J Hum Genet* 1978; **30**: 602.
22. Sandhoff K, Christomanou H. Biochemistry and genetics of gangliosidoses. *Hum Genet* 1979; **50**: 107.
23. Conzelmann E, Kytzia H-J, Navon R, Sandhoff K. Ganglioside GM₂ N-acetyl-beta-D-galactosaminidase activity in cultured fibroblasts of late-infantile and adult GM₂ gangliosidosis patients and of healthy probands with low hexosaminidase level. *Am J Hum Genet* 1983; **35**: 900.
24. Rapin I, Suzuki K, Suzuki K, Valsamis M. Adult (chronic) GM₂ gangliosidosis. Atypical spinocerebellar degeneration in a Jewish sibship. *Arch Neurol* 1976; **33**: 120.
25. Kaback M, Miles J, Yaffe M *et al.* Hexosaminidase A (Hex-A) deficiency in early adulthood: a new type of GM₂ gangliosidosis. *Am J Hum Genet* 1978; **30**: 31A.
26. Navon R, Argov Z, Brandt N, Sandbank U. Adult GM₂ gangliosidosis in association with Tay-Sachs disease: a new phenotype. *Neurology* 1981; **31**: 1397.
27. Dreyfus JC, Poenaru L, Svennerholm L. Absence of hexosaminidase A and B in a normal adult. *N Engl J Med* 1975; **292**: 61.
28. Dreyfus JC, Poenaru L, Vilbert M *et al.* Characterization of a variant of beta-hexosaminidase: 'Hexosaminidase Paris'. *Am J Hum Genet* 1977; **29**: 287.
29. Navon R, Argov Z, Frisch A. Hexosaminidase A deficiency in adults. *Am J Med Genet* 1986; **24**: 179.
30. Argov Z, Navon R. Clinical and genetic variations in the syndrome of adult GM₂ gangliosidosis resulting from hexosaminidase A deficiency. *Ann Neurol* 1984; **16**: 14.
31. Renshaw PF, Stern TA, Welch C *et al.* Electroconvulsive therapy treatment of depression in a patient with adult GM₂-gangliosidosis. *Ann Neurol* 1992; **31**: 342.
32. Petersen GM, Rotter JI, Cantor RM *et al.* The Tay-Sachs disease gene in North American Jewish populations: geographic variations and origin. *Am J Hum Genet* 1983; **35**: 1258.
33. Hanhart E. Über 27 Sippen mit infantiler amaurotischer Idiotie (Tay-Sachs). *Acta Genet Med Gemellol* 1954; **3**: 331.
34. Brzustowicz LM, Lehner T, Castilla LH *et al.* Genetic mapping of chronic childhood-onset spinal muscular atrophy to chromosome 5q112-133. *Nature* 1990; **344**: 540.
35. Klenk E. Über die ganglioside eine neue Gruppe von zuckerhaltigen Gehirnlipoiden. *Z Physiol Chem* 1942; **273**: 76.
36. Svennerholm L. Chemical structure of normal human brain and Tay-Sachs gangliosidoses. *Biochem Biophys Res Commun* 1962; **9**: 436.
37. Svennerholm L. Ganglioside metabolism. In: Florkin M, Stotz EH (eds). *Comprehensive Biochemistry*. Amsterdam: American Elsevier Publishing, 1970; **18**: 201.
38. Robinson D, Stirling JL. N-Acetyl-b-glucosaminidases in human spleen. *Biochem J* 1968; **107**: 301.
39. Suzuki K, Rapin I, Suzuki Y, Ishii N. Juvenile GM₂-gangliosidosis: clinical variant of Tay-Sachs disease or a new disease. *Neurology* 1970; **20**: 190.
40. Jatzkewitz H, Pilz H, Sandhoff K. The quantitative determination of gangliosides and their derivatives in different forms of amaurotic idiocy. *J Neurochem* 1965; **12**: 135.
41. O'Brien JS, Okada S, Chen A, Fillerup DL. Tay-Sachs disease: detection of heterozygotes and homozygotes by serum hexosaminidase assay. *N Engl J Med* 1970; **283**: 15.
42. Srivastava SK. Tay-Sachs and Sandhoff disease. In: Glew RH, Peters SP (eds). *Practical Enzymology of the Sphingolipidoses*. New York: Alan R Liss, 1977: 217.
43. Okada S, Veath ML, Leroy J, O'Brien JS. Ganglioside GM₂ storage diseases: hexosaminidase deficiencies in cultured fibroblasts. *Am J Hum Genet* 1971; **23**: 55.
44. Srivastava SK, Ansari NH, Hawkins LA, Wiktorowicz JE. Demonstration of cross-reacting material in Tay-Sachs disease. *Biochem J* 1979; **179**: 657.
45. Proia RL, Saravia E. Organization of the gene encoding the human beta-hexosaminidase alpha-chain. *J Biol Chem* 1987; **262**: 5677.
46. Akli S, Boue J, Sandhoff K *et al.* Collaborative study of molecular epidemiology of Tay-Sachs disease in Europe. *Eur J Hum Genet* 1993; **1**: 229.

47. Barker D, Schafer M, White R. Restriction sites containing CpG show a higher frequency of polymorphism in human DNA. *Cell* 1984; **36**: 131.
48. Paw BH, Wood LC, Neufeld EF. A third mutation at the CpG dinucleotide of codon 504 and a silent mutation at codon 506 of the HEX A gene. *Am J Hum Genet* 1991; **48**: 1139.
49. Mules EH, Dowling CE, Petersen MB *et al*. A novel mutation in the invariant AG of the acceptor splice site of intron 4 of the beta-hexosaminidase alpha-subunit gene in two unrelated American Black GM₂-gangliosidosis (Tay-Sachs disease) patients. *Am J Hum Genet* 1991; **48**: 1181.
50. Mules EH, Hayflick S, Miller CS *et al*. Six novel deleterious and three neutral mutations in the gene encoding the alpha-subunit of hexosaminidase A in non-Jewish individuals. *Am J Hum Genet* 1992; **50**: 834.
51. Myerowitz R, Hogikyan ND. Different mutations in Ashkenazi Jewish and non-Jewish French Canadians with Tay-Sachs disease. *Science* 1986; **232**: 1646.
52. Paw BH, Moskowitz SM, Uhrhammer N *et al*. Juvenile GM₂ gangliosidosis caused by substitution of histidine for arginine at position 499 or 504 of the alpha-subunit of beta-hexosaminidase. *J Biol Chem* 1990; **265**: 9452.
53. Akli S, Chelly J, Mezard C *et al*. A 'G' to 'A' mutation at position -1 of a 5' splice site in a late infantile form of Tay-Sachs disease. *J Biol Chem* 1990; **265**: 7324.
54. Paw BH, Kaback MM, Neufeld EF. Molecular basis of adult-onset and chronic GM₂ gangliosidoses in patients of Ashkenazi Jewish origin: substitution of serine for glycine at position 269 of the alpha-subunit of beta-hexosaminidase. *Proc Natl Acad Sci USA* 1989; **86**: 2413.
55. Kaback MM, Zeiger RS. Heterozygote detection in Tay-Sachs disease: a prototype community screening program for the prevention of genetic disorders. In: Volk BW, Aronson SM (eds). *Sphingolipidoses and Allied Disorders*. New York: Plenum Press, 1972: 613.
56. Kaback MM. Thermal fractionation of serum hexosaminidase: approaches to heterozygote detection and diagnosis of Tay-Sachs disease. In: Ginsberg V (ed.). *Methods in Enzymology*. New York: Academic Press, 1972: 862.
57. Lowden JA, Skomorowski MA, Henderson F, Kaback MM. Automated assays of hexosaminidases in serum. *Clin Chem* 1973; **19**: 1345.
58. Grebner EE, Wapner RJ, Barr MA, Jackson LG. Prenatal Tay-Sachs diagnosis by chorionic villi sampling. *Lancet* 1983; **2**: 286.
59. O'Brien JS. Pitfalls in the prenatal diagnosis of Tay-Sachs disease. In: Kaback M, Rimoin D, O'Brien JS (eds). *Tay-Sachs Disease: Screening and Prevention*. New York: Alan R Liss, 1977: 283.
60. Cantor RM, Lim JS, Roy C, Kaback MM. Sandhoff disease heterozygote detection: a component of population screening for Tay-Sachs disease carriers. I. Statistical methods. *Am J Hum Genet* 1985; **37**: 912.
61. Triggs-Raine BL, Mules EH, Kaback MM *et al*. A pseudodeficiency allele common in non-Jewish Tay-Sachs carriers: implications for carrier screening. *Am J Hum Genet* 1992; **51**: 793.
62. Triggs Raine BL, Archibald A, Gravel RA, Clarke JT. Prenatal exclusion of Tay-Sachs disease by DNA analysis. *Lancet* 1990; **335**: 1164.

Sandhoff disease/GM₂ gangliosidosis/deficiency of hexosaminidase A and B/hex-B subunit deficiency

Introduction	686	Treatment	691
Clinical abnormalities	686	References	691
Genetics and pathogenesis	689		

MAJOR PHENOTYPIC EXPRESSION

Progressive cerebral degeneration starting at six months of age, blindness, cherry red macular spots, hyperacusis, accumulation of GM₂ ganglioside, and deficiency of hexosaminidase A and B (Hex-A and Hex-B), resulting from mutation in the gene for Hex-B.

INTRODUCTION

The clinical phenotype of Sandhoff disease may be indistinguishable from that of Tay-Sachs disease ([Chapter 90](#)), but there may be hepatosplenomegaly in Sandhoff disease. The distinction between the two conditions was delineated by Sandhoff *et al.* [1] in 1968, in a patient who was unusual in that he stored ganglioside not only in the brain but also in other viscera. In contrast to patients with



Figure 91.1 SO: A 13-month-old child with Sandhoff disease in the tonic neck reflex position. He had spasticity and hyper-reflexic. Babinski responses were positive bilaterally and he had ankle clonus. The neck was hypotonic.

Tay-Sachs disease, the activity of total hexosaminidase was found to be deficient [1]. Hexosaminidase B is a glycoprotein homopolymer with four identical subunits; its structure is designated $\beta_2\beta_2$ [2, 3]. Hexosaminidase A is a heteropolymer of α and β subunits. Activity of the Hex-A and Hex-B isozymes are defective because of a defective β subunit. The disease has also been referred to as GM₂ gangliosidosis (variant O). The Hex-B gene is located on chromosome 5q13 [4]. Heterogeneity has been observed in the mutations in the gene for Hex-B [5]. Most mutations lead to the most severe infantile onset phenotype. The causative mutations in these patients tend to be deletions, nonsense mutations, or splice site mutations. The most common is a 16 kb deletion that includes the promoter, exons 1 to 5, and part of the intron [6].

CLINICAL ABNORMALITIES

It has often not been possible to distinguish Sandhoff disease from Tay-Sachs disease clinically [2, 3, 7–9]. The disorder tends to be suspected in non-Jewish patients with the Tay-Sachs phenotype. Individual patients appear normal at birth and appear to develop normally ([Figures 91.1, 91.2, 91.3, 91.4, 91.5, 91.6, 91.7, 91.8, and 91.9](#)) until four to nine months of age, when signs of motor weakness and hypotonia begin to become evident. Abilities that have been learned are progressively lost. These might



Figure 91.2 T0: There was no evidence of coarse features. Activities of hexosaminidase A and B were virtually completely deficient.



Figure 91.3 T0: At 18 months. Regression had begun after six months and all milestones were lost. She had an exaggerated startle to noise.

include the ability to grasp objects or to sit, crawl, or hold up the head. Patients never learn to walk. Many of these infants have doll-like faces with long eyelashes and fine hair, pale translucent skin and pink coloring. Cherry red macular spots (Figures 91.10 and 91.11) are seen bilaterally. Blindness is progressive and optic atrophy develops. Patients develop hyperacusis, or an exaggerated startle response to noise, which may be seen even quite early. The size of the head increases abnormally. Seizures are common; they develop some months after the onset of clear neurologic abnormality. They may be generalized or myoclonic. The electroencephalogram (EEG) also becomes



Figure 91.4 M0: A 17-month-old child with Sandhoff disease. She was flaccid and apathetic. Head circumference was increased and she had acoustic myoclonus to slight sound, as well as myoclonic and tonic seizures. Leukocyte hexosaminidase activity was zero.



Figure 91.5 M0: The vacant facial expression. She had bilateral cherry red spots.

progressively more abnormal. Visual loss is progressive and visual evoked potentials abnormal. Spasticity develops, and mental deterioration continues until the patient is rigid, decerebrate, and completely blind. Computed tomography (CT) scan may reveal the Turkish moustache sign (Figure

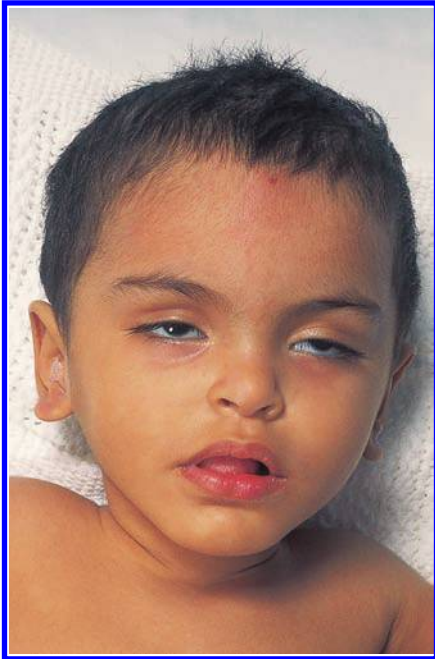


Figure 91.6 TO: Indicating the similar facial expression.



Figure 91.8 MSO: An 11-month-old with Sandhoff disease had a similar facial appearance and cherry red spots.



Figure 91.7 AMH: This 14-month-old boy with Sandhoff disease had a similar facial appearance, cherry red macular spots, acoustic myoclonus and no detectable leukocyte hexosaminidase activity.

91.12). Alimentation requires tube feeding. Death usually occurs between the ages of one and four years, most often from bronchopneumonia or aspiration.

At autopsy, the visceral organs may be somewhat heavier than those of patients with Tay-Sachs disease [7]. Visceral storage may be evident in lipid-laden histiocytes [2]. Some

patients may have clinical hepatosplenomegaly, but most do not. Renal tubular cells show lipid deposits. Foamy histiocytes may be seen in bone marrow aspirates. In the brain there is a typical neuronal lipidosis. Membranous cytoplasmic bodies seen by electronmicroscopy are identical to those characteristic of Tay-Sachs disease. Vacuolated cells and lamellar inclusions are demonstrable by conjunctival biopsy.

A small number of variant later onset forms of Sandhoff disease have been observed. They have variously been referred to as juvenile, subacute or adult – chronic, but now that there are molecular distinctions it is likely that there will be a spectrum of phenotype. The majority of these variants present between two and ten years of age, most often with ataxia or incoordination [10–15]. There may be choreoathetosis or dystonia. Neurodegeneration is progressive, and seizures and spasticity develop. There may be cherry red spots, or more commonly retinitis pigmentosa and optic atrophy. By 10–15 years, the patient is blind and decerebrate, as in the infantile patient, and death shortly ensues. One patient referred to as having a juvenile form of Sandhoff disease [11] developed slurred speech, ataxia and some mental deterioration at five years of age. By ten years, he had spasticity, but the optic fundi were normal.

Adult onset patients may have psychiatric symptoms. Ataxia and an apparent spinocerebellar picture may be evident [10–15]. Dysarthria may be severe. The disorder may be very slowly progressive. In late onset patients, typical membranous cytoplasmic bodies have been reported in the myenteric plexus [16].



Figure 91.9 (A and B) A boy with Sandhoff disease at 18 and 19 months indicating the change in expression with progressive degeneration. He was blind and had cherry red spots at the time of the first photograph.



Figure 91.10 SO: The cherry red spot.



Figure 91.11 TO: The cherry red spot of an 18-month-old with Sandhoff disease. A cousin died at 17 months with a similar picture. Two brothers married two sisters. Hexosaminidase activity of both parents was in the heterozygote range.



Figure 91.12 Computed tomographic scan of the brain of a patient with Sandhoff disease indicating the Turkish moustache sign.

GENETICS AND PATHOGENESIS

Patients with Sandhoff disease accumulate GM₂ ganglioside in the brain [1]. The amounts found are 100–300 times the normal concentrations and quite similar to those of Tay-Sachs disease. In contrast to patients with Tay-Sachs disease, these patients also accumulate globoside, the

common neutral glycolipid of erythrocyte and renal membranes, which has the same amino terminal sugar as GM₂ ganglioside, N-acetyl galactosamine, in extraneural tissues, especially the liver, kidney, and spleen [17–20]. In the brain there is storage of GM₂; in addition, the asialo derivative of GM₂ (GA₂) accumulates, and this too is a difference from Tay-Sachs disease. Globoside may be demonstrated in urinary sediments and plasma [18]. The stored compounds are all structurally related. The asialo derivative differs from GM₂ in the absence of the N-acetylneuraminic side chain, whereas globoside contains an extra galactose moiety. GA₂ is found in the brain in Sandhoff disease in amounts 100 times normal [17]. Oligosaccharides and glycopeptides, which have a glycosidically bound N-acetylhexosamine, accumulate in various tissues, and they are excreted in the urine [21, 22], providing a readily accessible approach to diagnosis.

The Sandhoff disease is characterized by the lack not only of hexosaminidase A, but also of hexosaminidase B [7, 20–24]. The enzyme defect has been demonstrated with natural substrates, including GM₂, GA₂ and globoside, as well as with the artificial substrates nitrophenylacetylglucosaminide and 4-methylumbelliferyl-N-acetylglucosamine [1, 7]. The deficiency is present in all tissues of the body. It is readily assessed in serum, leukocytes, and cultured fibroblasts [17]. In the classic infantile patients, the activity of each enzyme is about 1–3 percent of normal [17]. Distinctions among the variant GM₂ gangliosidoses cannot be done well using the usual assays with artificial substrate, because there is a poor correlation between severity of clinical phenotype and degree of enzyme residual activity [25].

Each of the Hex-A and Hex-B enzymes has a molecular weight of about 100,000. The subunits are about 25,000 daltons. Both hexosaminidases cleave the lipids globoside and GA₂, but only hexosaminidase A hydrolyzes GM₂. The β chain is coded for on chromosome 5, while the α chain is determined by a locus on chromosome 15. Cells from patients with Sandhoff disease lack the β chains [26]. In some examples of Sandhoff disease the residual hexosaminidase in liver has been shown to have an increased Km, and pH optimum, indicating that there is a structural gene alteration [23]. In somatic cell hybrids, there was independent segregation of hexosaminidase A and B, consistent with their loci on two different chromosomes [27, 28]. Hybridization of fibroblasts from a patient with Tay-Sachs disease with those of a patient with Sandhoff disease revealed complementation in which hexosaminidase activity appeared, although it was present in neither parental strain [29]. Correction of hexosaminidase A activity represented provision of the α subunit from the Sandhoff fibroblasts and the β unit from the Tay-Sachs cells to form a hybrid heteropolymer [29–31].

Sandhoff disease is transmitted as an autosomal recessive trait. The gene for the disease appears to be unusually prevalent in Lebanon [32]. It is also high in an

area north of Cordoba in Argentina [33]. In Saudi Arabia [34], it is one of four frequently encountered lysosomal storage diseases. Unlike the others, Sandhoff disease was tribal in the sense that half of the patients were of one large tribe. In California there is an increased frequency among Hispanic people of Mexican or Central American origin [35]. Most recent estimates [36] of carrier frequency have yielded a frequency of one in 278 for carriers of the Sandhoff gene in non-Jews and one in 500 in Jews. This would yield a frequency of infants born with the disease of one in 300,000 non-Jews and one in a million Jews.

The Hex-B gene contains 14 exons and spans approximately 45 kb [37, 38]. There is extensive homology with the Hex-A gene, as there is between the two α and β proteins. The mutations observed in Sandhoff disease have been heterogeneous [11, 37–39]. Some yield subunits that are cross-reacting material (CRM) positive; others are CRM negative. In the classic infantile Sandhoff disease, there have been a number of partial deletions; there may be normal or reduced amount of mRNA, but the activity of hexosaminidase A is always essentially undetectable [5]. In a patient with later onset at five years [11], whose variant was referred to as hexosaminidase Paris [40–42], hexosaminidase B activity was deficient, but there was preservation of some activity of hexosaminidase A. Another variant had considerable activity of both isozymes in serum but marked reduction in tissues [38]. In general, among the so-called juvenile variants, hexosaminidase A activity has been expressed at 1–3 percent of control [43].

Mutations identified in classic infantile patients have usually been major alterations [44]. In addition to the 16 kb deletion involving the promoter and exons 1 to 5 [6], a 50 kb deletion was found in a single family [45]. At least one splicing mutation led to almost complete absence of mRNA.

Among later onset patients, many were compound heterozygotes, such as I207V and Y456S [46]. In a family in which there was compound heterozygosity for P417L and the severe 16 kb deletion which, when homozygous, leads to the classic infantile disease, there was late onset presentation in the 51-year-old proband and four asymptomatic patients, 51–61 years of age [47]. In the hexosaminidase Paris, the Hex-B minus, Hex-A plus phenotype, a duplication straddling intron 13 and exon 14 generated an alternative splice site and caused an in-frame insertion of 18 nucleotides for the mRNA. The second allele was not known. The 16 kb deletion has been observed in French and French Canadian patients. Among the Argentine patients, there were two null mutations, a G to A transition (IVS 2 + 1) and a four base deletion (784del4) [48].

Detection of heterozygous carriers is possible by enzyme assay, which reveals amounts of hexosaminidase A and B in leukocytes, skin, cultured fibroblasts, and serum that are intermediate between normal and patient concentrations [49–53]. Heterozygotes have been reported [9] in whom the activity of the A isozyme was present, but the B isozyme was less than 20 percent of normal. In heterozygotes, the

B isozyme was more thermolabile than normal [53], indicating the presence of a heteropolymer containing mutant and normal β chains. Intrauterine diagnosis has been accomplished by assay of cultured amniocytes [54–56]. The detection of N-acetylgalactosaminyloligosaccharides in amniotic fluid has been used for prenatal diagnosis, as it has in the urine for postnatal diagnosis [57, 58]. In a family in which the mutation is known, mutational analysis is the method of choice for prenatal diagnosis and for heterozygote detection.

TREATMENT

The treatment of Sandhoff disease is entirely supportive, but a variety of experimental approaches to therapy are being explored. An animal model for Sandhoff disease in cats and a knockout mouse permit rational studies of therapy [59–61]. These include enzyme replacement, bone marrow transplantation, and gene therapy. In the mouse, bone marrow transplantation prolonged lifespan from four or five months to eight months and appeared to slow neurologic degeneration, but there was no improvement in storage of glycolipid in brain or neuronal pathology [61]. Bone marrow transplantation in a patient with Sandhoff disease appeared to be without beneficial effect [62].

Substrate deprivation therapy in which an inhibitor lowers the synthesis of glycosphingolipid has been employed in the Sandhoff mouse, with N-butyldeoxynojirimycin (NB-DNJ), an inhibitor of glucosylceramide synthase [63]. Treatment prolonged lifespan and reduced storage of GM₂ and GA₂ in brain. It is expected that treatment would be of greater utility in later-onset phenotypes, rather than in the infantile form of the disease. Therapy should potentially be of use in all three forms of GM₂ gangliosidosis (Chapters 90 and 92), Gaucher disease (Chapter 93), GM₁ gangliosidosis (Chapter 89), and Fabry disease (Chapter 88).

REFERENCES

- Sandhoff K, Andrae U, Jatzkewitz H. Deficient hexosaminidase activity in an exceptional case of Tay-Sachs disease with additional storage of kidney globoside in visceral organs. *Life Sci* 1968; **7**: 283.
- Sandhoff K. Sphingolipidoses. *J Clin Pathol* 1974; **27**(Suppl. 8): 94.
- Sandhoff K, Christomanou H. Biochemistry and genetics of gangliosidoses. *Hum Genet* 1979; **50**: 107.
- Fox MF, DuToit DL, Warnich L, Retief AE. Regional localization of alpha-galactosidase (GLA) to Xpter q22 hexosaminidase B (HEXB) to 5q13 → qter and arylsulfatase B (ARSB) to 5pter → q13. *Cytogenet Cell Genet* 1984; **38**: 45.
- O'Dowd BF, Klavins MH, Willard HF *et al.* Molecular heterogeneity in the infantile and juvenile forms of Sandhoff disease (O-variant GM₂ gangliosidosis). *J Biol Chem* 1986; **261**: 12680.
- Neote K, McInnes B, Mahuran DJ, Gravel RA. Structure and distribution of an Alu-type deletion mutation in Sandhoff disease. *J Clin Invest* 1990; **86**: 1524.
- Sandhoff K, Harzer K, Wassle W, Jatzkewitz H. Enzyme alterations and lipid storage in three variants of Tay-Sachs disease. *J Neurochem* 1971; **18**: 2469.
- Dolman CL, Chang E, Duke RJ. Pathologic findings in Sandhoff disease. *Arch Pathol* 1973; **96**: 272.
- Costa TR, Pampols T, Gonzalez-Sastre F *et al.* Enfermedad de Sandhoff (Gangliosidosis GM₂ tipo II) Presentacion de un case con estudio clinico y bioquimico Investigacion de portadores. *Ann Exp Pediat* 1984; **20**: 146.
- Johnson WG. The clinical spectrum of hexosaminidase deficiency disease. *Neurology* 1981; **31**: 1453.
- MacLeod PM, Wood S, Jan JE *et al.* Progressive cerebellar ataxia psychomotor retardation and hexosaminidase deficiency in a 20-year-old child: juvenile Sandhoff disease. *Neurology* 1977; **27**: 571.
- Brett EM, Ellis RB, Haas L *et al.* Late onset GM₂ gangliosidosis: clinical pathological and biochemical studies in eight patients. *Arch Dis Child* 1973; **48**: 775.
- Wood S, MacDougall BG. Juvenile Sandhoff disease: some properties of the residual hexosaminidase in cultured fibroblasts. *Am J Hum Genet* 1976; **28**: 489.
- Kolodny E, Raghavan S. Hexosaminidase mutations not of the Tay-Sachs type produce unusual clinical variants. *Trends Neurosci* 1983; **6**: 16.
- Navon R. Molecular and clinical heterogeneity of adult GM₂ gangliosidosis. *Dev Neurosci* 1991; **13**: 295.
- Rubin M, Karpati G, Wolfe LS *et al.* Adult onset motor neuronopathy in the juvenile type of hexosaminidase A and B deficiency. *J Neurol Sci* 1988; **87**: 103.
- Okada S, McCrean M, O'Brien JS. Sandhoff's disease (GM₂ gangliosidosis type 2): clinical chemical and enzyme studies in five patients. *Pediatr Res* 1972; **6**: 606.
- Krivit W, Desnick RJ, Lee J *et al.* Generalized accumulation of neutral glycosphingolipids with GM₂ ganglioside accumulation in the brain. *Am J Med* 1972; **52**: 763.
- Snyder PD, Krivit W, Sweeley CC. Generalized accumulation of neutral glycosphingolipids with GM₂ ganglioside accumulation in the brain. *J Lipid Res* 1972; **13**: 128.
- Sandhoff K, Harzer K. Total hexosaminidase deficiency in Tay-Sachs disease (variant O). In: Hers HG, Van Hoof F (eds). *Lysosomes and Storage Diseases*. New York: Academic Press, 1973: 345.
- Cantz M, Kresse H. Sandhoff disease: defective glycosaminoglycan catabolism in cultured fibroblasts and its correction by β -N-acetylhexosaminidase. *Eur J Biochem* 1974; **47**: 581.
- Strecker G, Montreuil J. Glycoproteins et glycoproteinoses. *Biochimie* 1979; **61**: 1199.
- Tateson R, Bain AD. GM₂ gangliosidoses. Consideration of the genetic defects. *Lancet* 1971; **2**: 612.
- Suzuki Y, Jacob JC, Suzuki K, Kutty KM. GM₂ gangliosidosis with total hexosaminidase deficiency. *Neurology* 1971; **21**: 313.
- Geiger B, Arnon R. Chemical characterization and subunit

- structure of human N-acetylhexosaminidases A and B. *Biochemistry* 1976; **15**: 3484.
26. Proia RL, Neufeld EF. Synthesis of β -hexosaminidase in cell-free translation and intact fibroblasts: an insoluble precursor alpha chain in a rare form of Tay-Sachs disease. *Proc Natl Acad Sci USA* 1982; **79**: 6360.
27. Gilbert F, Kucherlapati R, Creagan RP et al. Tay-Sachs and Sandhoff's diseases: the assignment of genes for hexosaminidases A and B to individual human chromosomes. *Proc Natl Acad Sci USA* 1975; **72**: 263.
28. Lalley PA, Rattazzi MC, Shows TB. Human β -d-N-acetyl-hexosaminidase A and B: expression and linkage relationships in somatic cell hybrids. *Proc Natl Acad Sci USA* 1974; **71**: 1569.
29. Thomas GH, Taylor HA, Miller CS et al. Genetic complementation after fusion of Tay-Sachs and Sandhoff cells. *Nature* 1974; **250**: 580.
30. Galjaard H, Hoogeveen A, De Wit-Verbeek HA et al. Tay-Sachs and Sandhoff's disease: intergenic complementation after somatic cell hybridization. *Exp Cell Res* 1974; **87**: 444.
31. Srivastava SK, Beutler E. Studies on human β -d-acetyl-hexosaminidase. III Biochemical genetics of Tay-Sachs and Sandhoff's disease. *J Biol Chem* 1974; **249**: 2054.
32. Der Karloustian VM, Khoury MJ, Hallal R et al. Sandhoff disease: a prevalent form of infantile GM₂ gangliosidosis in Lebanon. *Am J Hum Genet* 1981; **33**: 85.
33. De Kremer RD, De Levstein IM. Enfermedad de Sandhoff O gangliosidosis GM₂, tipo 2. Alta frecuencia del gen en una poblacion criolla. *Medicina (Buenos Aires)* 1980; **40**: 55.
34. Ozand PT, Gascon G, Al Aqeel A et al. Prevalence of different types of lysosomal storage diseases in Saudi Arabia. *J Inherit Metab Dis* 1990; **13**: 849.
35. Cantor RM, Roy C, Lim JST, Kaback MM. Sandhoff disease heterozygote detection: a component of population screening for Tay-Sachs disease carriers. II Sandhoff disease gene frequencies in American Jewish and non-Jewish populations. *Am J Hum Genet* 1987; **41**: 16.
36. Kaback M. *Summary of Worldwide Tay-Sachs Disease Screening and Detection*. Los Angeles: University of California, 1988.
37. Proia RL. Gene encoding the human beta-hexosaminidase beta chain: extensive homology of intron placement in the alpha- and beta-chain genes. *Proc Natl Acad Sci USA* 1988; **85**: 1883.
38. Neote K, Bapat B, Dumbrille Ross A et al. Characterization of the human HEXB gene encoding lysosomal beta-hexosaminidase. *Genomics* 1988; **3**: 279.
39. Lane AB, Jenkins T. Two variant hexosaminidase β -chain alleles segregating in a South African family. *Clin Chim Acta* 1978; **87**: 219.
40. Dreyfus JC, Peonaru L, Svennerholm L. Absence of hexosaminidase A and B in a normal adult. *N Engl J Med* 1975; **292**: 61.
41. Dreyfus JC, Poenaru L, Vibert M et al. Characterization of a variant of β -hexosaminidase: 'Hexosaminidase Paris'. *Am J Hum Genet* 1977; **29**: 287.
42. Dlott B, D'Azzo A, Quon DVK, Neufeld EF. Two mutations produce intron insertion in mRNA and elongated beta-subunit of human beta hexosaminidase. *J Biol Chem* 1990; **265**: 17921.
43. Conzelmann E, Kytzia H-J, Navon R, Sandhoff K. Ganglioside GM₂ N-acetyl-beta-D-galactosaminidase activity in cultured fibroblasts of late-infantile and adult GM₂ gangliosidosis patients and of healthy probands with low hexosaminidase levels. *Am J Hum Genet* 1983; **35B**: 900.
44. Neufeld EF. Natural history and inherited disorders of a lysosomal enzyme beta-hexosaminidase. *J Biol Chem* 1989; **264**: 10927.
45. Zhang ZX, Wakamatus N, Akerman BR et al. A second large deletion in the HEXB gene in a patient with infantile Sandhoff disease. *Hum Mol Genet* 1995; **4**: 777.
46. Banerjee P, Siciliano L, Oliveri D et al. Molecular basis of an adult form of beta-hexosaminidase B deficiency with motor neuron disease. *Biochem Biophys Res Commun* 1991; **181**: 108.
47. McInnes B, Potier M, Wakamatsu N et al. An unusual splicing mutation in the HEXB gene is associated with dramatically different phenotypes in patients from different racial backgrounds. *J Clin Invest* 1992; **90**: 306.
48. Brown CA, McInnes B, De Kremmer RD, Mahuran DJ. Characterization of two HEXB gene mutations in Argentinean patients with Sandhoff disease. *Biochim Biophys Acta* 1992; **1180**: 91.
49. Harzer K. Inheritance of the enzyme deficiency in three neuro lipidoses: variant O of Tay-Sachs disease (Sandhoff's disease), classic Tay-Sach's disease and metachromatic leukodystrophy. Identification of the heterozygous carriers. *Humangenetik* 1973; **20**: 1.
50. Harzer K, Sandhoff K, Schall H, Kollman F. Enzymatische untersuchungen im blut von ubertragern einer variante der Tay-Sach's chen erkrankung (variante O). *Klin Wschr* 1971; **49**: 1189.
51. Kolodny EH. Sandhoff's disease: studies on the enzyme defect in homozygotes and detection of heterozygotes. In: Volk BW, Aronson SM (eds). *Sphingolipidoses and Allied Disorders*. New York: Plenum Press, 1972: 321.
52. Lowden JA, Ives EJ, Keene DL et al. Carrier detection in Sandhoff's disease. *Am J Hum Genet* 1978; **30**: 38.
53. Lowden JA. Evidence for a hybrid hexosaminidase isoenzyme in heterozygotes for Sandhoff disease. *Am J Hum Genet* 1979; **31**: 281.
54. Desnick RJ, Krivit W, Sharp HL. *In utero* diagnosis of Sandhoff's disease. *Biochem Biophys Res Commun* 1973; **51**: 20.
55. Kaback MM, Howell RR. Heterozygote detection and prenatal diagnosis of lysosomal diseases. In: Hers HG, Van Hoof F (eds). *Lysosomes and Storage Diseases*. New York: Academic Press, 1973: 599.
56. Harzer K, Stengel-Rutkowski S, Gley EO et al. Prenatale diagnose der GM₂ gangliosidose type 2 (Sandhoff-Jatzkowitsz-Krankheit). *Dtsch Med Wschr* 1975; **100**: 106.
57. Warner TG, De Kremer RD, Applegarth D, Mock AK. Diagnosis and characterization of GM₂ gangliosidosis type II (Sandhoff disease) by analysis of the accumulating N-acetyl-glucosaminyl oligosaccharides with high performance liquid chromatography. *Clin Chim Acta* 1986; **154**: 151.
58. Warner TG, Turner MW, Toone JR, Applegarth D. Prenatal diagnosis of infantile GM₂ gangliosidosis type II

- (Sandhoff disease) by detection of N-acetylglucosaminyl-oligosaccharides in amniotic fluid with high-performance liquid chromatography. *Prenat Diagn* 1986; **6**: 393.
59. Cork LC, Munnell JF, Lorenz MD *et al.* GM₂ ganglioside storage disease in cats with beta-hexosaminidase deficiency. *Science* 1977; **196**: 1014.
60. Bikker H, Van Den Berg FM, Wolterman RA *et al.* Demonstration of a Sandhoff disease-associated autosomal 50-kb deletion by filed inversion gel electrophoresis. *Hum Genet* 1989; **81**: 287.
61. Norflus F, Tifft CJ, McDonald MP *et al.* Bone marrow transplantation prolongs life span and ameliorates neurologic manifestations in Sandhoff disease mice. *J Clin Invest* 1998; **101**: 1881.
62. Hoogerbrugge PM, Brouwer OF, Bordigoni P *et al.* Allogeneic bone marrow transplantation for lysosomal storage disease. The European Group for Bone Marrow Transplantation. *Lancet* 1995; **345**: 1398.
63. Jeyakumar M, Butters TD, Cortina-Borja M *et al.* Delayed symptom onset and increased life expectancy in Sandhoff-disease mice treated with N-butyldeoxynojirimycin. *Proc Natl Acad Sci USA* 1999; **96**: 6388.

GM₂ activator deficiency/GM₂ gangliosidosis – deficiency of the activator protein

Introduction	694	Treatment	696
Clinical abnormalities	694	References	696
Genetics and pathogenesis	695		

MAJOR PHENOTYPIC EXPRESSION

Progressive cerebral degeneration indistinguishable clinically from Tay-Sachs disease, with blindness, cherry red macular spots, hyperacusis, accumulation of GM₂ ganglioside, and deficiency of the GM₂ activator.

INTRODUCTION

GM₂ activator deficiency was discovered in 1978 by Conzelmann and Sandhoff [1] in a patient who accumulated GM₂ ganglioside, but was not deficient in hexosaminidase A activity. The disorder has been referred to as the AB variant of GM₂ gangliosidosis [2]; with Tay-Sachs and Sandhoff diseases there are now three nonallelic variants that cause GM₂ gangliosidosis.

The GM₂ activator protein is one of a family of sphingolipid activator proteins (SAPs) which though nonenzymatic are required for sphingolipid degradation [3]. The GM₂ activator forms a 1:1 water-soluble complex with lipid substrates [4]; it also interacts specifically with the hexosaminidase A enzyme [5] and in these ways promotes the catalytic degradation of GM₂ ganglioside. The gene for the activator protein is located on chromosome number 5 [6] at q31 [6, 7]. Each of the point mutations described to date leads to premature degradation of the activator protein and storage of GM₂ ganglioside [8–12].

CLINICAL ABNORMALITIES

The clinical phenotype of this disease is identical to that of Tay-Sachs disease [13–17]. In some patients, onset and progress may be a little slower than in the classic Tay-Sachs patient. Patients appear normal at birth and develop normally for the first six months, then they begin to lose

acquired milestones. Neurologic degeneration is rapidly progressive. Tendon reflexes are brisk and the Babinski response positive. Patients may become macrocephalic. By one year, they are usually blind, rigid, and decerebrate (Figures 92.1 and 92.2). They have typical hyperactive startle responses to noise. Cherry red spots are readily visible in the ocular fundi (Figure 92.3). Seizures are common. One patient [12] had hepatomegaly, but no others had any suggestion of visceral storage. Death usually supervenes by one to four years of age.



Figure 92.1 Early photograph of the girl in Figure 92.2 documenting unremarkable early development, which appeared normal at both six and ten months. The parents were first cousins.



Figure 92.2 A two-year-old Saudi girl with GM₂ gangliosidosis on the basis of deficiency of the activator protein. Her appearance was reminiscent of a Dresden china doll. She had developed well for eight months and then regressed. At two, she was blind and lay in a frog-leg position. Tone was increased and reflexes brisk. Babinski responses were positive.

Gross pathological examination of the brain has been described as normal [13–15, 17]. Histologic examination reveals typical neuronal storage throughout the brain along with demyelination of the subcortical white matter. Golgi preparations reveal increased numbers and size of meganeurites and atrophy of the perisomatic dendritic system [18]. Electron microscopy reveals the typical lamellar membranous cytoplasmic bodies in neurons, as in Tay-Sachs disease. Some neurons have zebra bodies and other inclusions. Inclusions in glial cells provide a difference from Tay-Sachs disease [15].

GENETICS AND PATHOGENESIS

In contrast to Tay-Sachs disease, patients studied to date have all been non-Jewish. Inheritance appears to be autosomal recessive. Our patient was the product of a first-cousin mating. Spanish parents were also first cousins [12]. Knowledge of the mutation should permit prenatal diagnosis and heterozygote detection.

Mutation at the GM₂ activator locus has been observed to result in extreme reduction of the activator protein [19]. Amounts of cross-reacting material (CRM) have ranged from 0 to 5 percent of control. The protein found in one

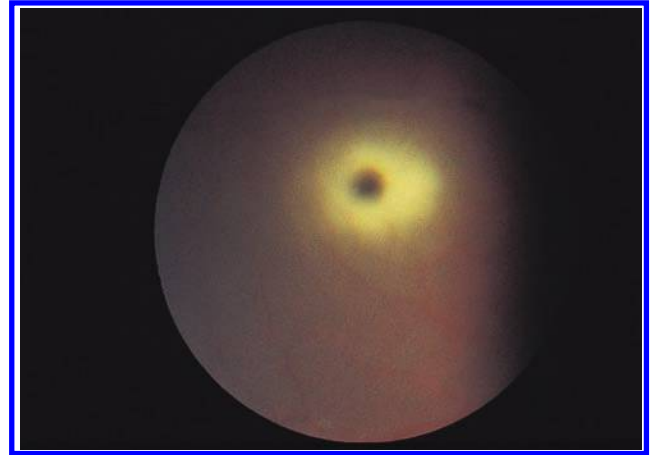


Figure 92.3 The cherry red or reddish black spot of macular degeneration. The activator protein was studied by Dr Sandhoff, who found no immunoprecipitable material and no utilization of the GM₂ ganglioside by cultured fibroblasts.

patient's fibroblasts was appreciably larger, at 26 kDa, than the normal activator protein. In this respect, it resembled the precursor form of the protein [20], and it was secreted into the medium.

The disease has generally been suspected when assay of hexosaminidase A or A and B with the usual artificial substrates gives normal results in a patient with otherwise typical infantile GM₂ gangliosidosis [1, 17, 21]. Degradation of the natural GM₂ ganglioside requires the activator protein. The hexosaminidase isozymes A and B are present in normal or elevated amounts [22].

The GM₂ activator protein is a small, acidic, monomeric protein containing 162 amino acids [23–25]. It is synthesized as a larger precursor protein and cleaved to the mature form; it is a glycoprotein containing a carbohydrate chain linked to the N of asparagine 32 [20, 24], and it is situated in the lysosomes [19]. The activator binds to substrate and hexosaminidase A, which then catalyzes the cleavage of the oligosaccharides of GM₂ and the glycolipid GA₂ [26].

The concentration of the activator, and the presence of a deficient content can be determined by an enzyme-linked immunoassay [25]. Detection of CRM-positive mutant proteins must be detected by assay of the degradation of GM₂ by fibroblasts [27].

The cDNA for the activator protein has been isolated and sequenced [28, 29]. It contains four exons over approximately 2.4 kb [30]. Among mutations identified, a T-to-C transition at nucleotide 412 resulted in substitution of an arginine for cysteine at position 107 [8, 9]. Transfection of cells with the mutant cDNA yielded low levels of the activator protein, indicating that the mutation

is responsible for the disease phenotype. A Saudi patient was homozygous for a 3 bp deletion, which deleted lysine 88 [12]. A Spanish patient was homozygous for a single base deletion which caused a frameshift [12]. Expression of the cDNA in *Escherichia coli* yielded a carbohydrate-free fusion protein that was taken up by cells and restored the activity of mutant fibroblasts to degrade GM₂ ganglioside [30]. This should permit the isolation of quantities of protein that would permit x-ray crystallography. It raises the possibility of therapy.

TREATMENT

Only supportive treatment is available.

REFERENCES

- Conzelmann E, Sandhoff K. AB variant of infantile GM₂-gangliosidosis: deficiency of a factor necessary for stimulation of hexosaminidase A-catalyzed degradation of ganglioside GM₂ and glycolipid GA₂. *Proc Natl Acad Sci USA* 1978; **75**: 3979.
- Sandhoff K, Harzer K, Wassle W, Jatzkewitz H. Enzyme alterations and lipid storage in three variants of Tay-Sachs disease. *J Neurochem* 1971; **18**: 2469.
- Sonderfeld S, Conzelmann E, Schwarzmann G *et al*. Incorporation and metabolism of ganglioside GM₂ in skin fibroblasts from normal and GM₂ gangliosidosis subjects. *Eur J Biochem* 1985; **149**: 247.
- Conzelmann E, Burg J, Stephan G, Sandhoff K. Complexing of glycolipids and their transfer between membranes by the activator protein for lysosomal ganglioside GM₂ degradation. *Eur J Biochem* 1982; **123**: 455.
- Kytzia H-J, Sandhoff K. Evidence for two different active sites on human hexosaminidase A: interaction of GM₂ activator protein with hexosaminidase A. *J Biol Chem* 1985; **260**: 7568.
- Burg J, Conzelmann E, Sandhoff K *et al*. Mapping of the gene coding for the human GM₂ activator protein to chromosome 5. *Ann Hum Genet* 1985; **49**: 41.
- Heng HH, Xie B, Shi XM *et al*. Refined mapping of the GM₂ activator protein (GM₂A) locus to 5q313-331 distal to the spinal muscular atrophy locus. *Genomics* 1993; **18**: 429.
- Schroder M, Schnabel D, Suzuki K, Sandhoff K. A mutation in the gene of a glycolipid-binding protein (GM₂ activator) that causes GM₂-gangliosidosis variant AB. *FEBS Lett* 1991; **290**: 1.
- Xie B, Wang W, Mahuran DJ. A Cys138-to-Arg substitution in the GM₂ activator protein is associated with the AB variant form of GM₂ gangliosidosis. *Am J Hum Genet* 1992; **50**: 1046.
- Xie B, Rigat B, Smijanac-Georgijev N *et al*. Biochemical characterization of the Cys138Arg substitution associated with the AB variant form of GM₂ gangliosidosis: evidence that Cys138 is required for the recognition of the GM₂ activator/GM₂ ganglioside complex by beta-hexosaminidase A. *Biochemistry* 1998; **37**: 814.
- Schroder M, Schnabel D, Hurwitz R *et al*. Molecular genetics of GM₂-gangliosidosis AB variant: a novel mutation and expression in BHK cells. *Hum Genet* 1993; **92**: 437.
- Schepers U, Glombitza GJ, Lemm T *et al*. Molecular analysis of a GM₂-activator deficiency in two patients with GM₂-gangliosidosis AB variant. *Am J Hum Genet* 1996; **59**: 1048.
- Goldman JE, Yamanaka T, Rapin I *et al*. The AB-variant of GM₂ gangliosidosis. Clinical biochemical and pathological studies of two patients. *Acta Neuropathol (Berl)* 1980; **52**: 189.
- Kolodny EH, Pruszkow IW, Moser HW *et al*. GM₂ gangliosidosis without deficiency in the artificial substrate cleaving activity of hexosaminidase A and B. *Neurology* 1973; **23**: 427.
- De Baeque CM, Suzuki K, Rapin I *et al*. GM₂ gangliosidosis AB variant. Clinico-pathological study of a case. *Acta Neuropathol (Berl)* 1975; **33**: 207.
- Sakuraba H, Itoh K, Shimmoto M *et al*. GM₂ gangliosidosis AB variant – clinical and biochemical studies of a Japanese patient. *Neurology* 1999; **52**: 372.
- Hechtman P, Gordon BA, Ng Ying Kim NMK. Deficiency of the hexosaminidase A activator protein in a case of GM₂ gangliosidosis; variant AB. *Pediatr Res* 1982; **16**: 217.
- Purpura DP. Ectopic dendritic growth in mature pyramidal neurons in human ganglioside storage disease. *Nature* 1978; **276**: 520.
- Banerjee A, Burg J, Conzelmann E *et al*. Enzyme-linked immunosorbent assay for the ganglioside GM₂-activator protein. Screening of normal human tissues and body fluids of tissues of GM₂ gangliosidosis, and for its subcellular localization. *Hoppe-Seyler's Z Physiol Chem* 1984; **365**: 347.
- Burg J, Banerjee A, Sandhoff K. Molecular forms of GM₂-activator protein. A study on its biosynthesis in human skin fibroblasts. *Biol Chem Hoppe-Seyler* 1985; **366**: 887.
- Hirabayashi Y, Li Y-T, Li S-C. The protein activator specific for the enzymic hydrolysis of GM₂ ganglioside in normal human brain and in brains of three types of GM₂ gangliosidosis. *J Neurochem* 1983; **40**: 168.
- Conzelmann E, Sandhoff K, Nehrkorn H *et al*. Purification, biochemical and immunological characterization of hexosaminidase A from variant AB of infantile GM₂ gangliosidosis. *Eur J Biochem* 1978; **84**: 27.
- Conzelmann E, Sandhoff K. Purification and characterization of an activator protein for the degradation of glycolipids GM₂ and GA₂ by hexosaminidase A. *Hoppe-Seyler's Z Physiol Chem* 1979; **360**: 1837.
- Furst W, Schubert J, Machleidt W *et al*. The complete amino-acid sequences of human ganglioside GM₂-activator protein and cerebroside sulfate activator protein. *Eur J Biochem* 1990; **19**: 709.
- Wu YY, Sonnino S, Li YT, Li SC. Characterization of an alternatively spliced GM₂ activator protein, GM₂A protein. An activator protein which stimulates the enzymatic hydrolysis of N-acetylneuraminic acid, but not N-acetylgalactosamine, from GM₂. *J Biol Chem* 1996; **271**: 10611.
- Meier EM, Schwarzmann G, Furst W, Sandhoff K. The human GM₂ activator protein. *J Biol Chem* 1991; **266**: 1879.
- Leinekugel P, Michel S, Conzelmann E, Sandhoff K. Quantitative correlation between the residual activity of

- beta-hexosaminidase A and arylsulfatase A and the severity of the resulting lysosomal storage disease. *Hum Genet* 1992; **88**: 513.
28. Schroder M, Klima H, Nakano T *et al.* Isolation of a cDNA encoding in the human GM₂ activator protein. *FEBS Lett* 1989; **251**: 197.
29. Klima H, Tanaka A, Schnabel D *et al.* Characterization of full-length cDNA and the gene coding for the human GM₂-activator protein. *FEBS Lett* 1991; **289**: 260.
30. Klima H, Klein A, Van Echten G *et al.* Over-expression of a functionally active human GM₂-activator protein in *Escherichia coli*. *Biochem J* 1993; **292**: 571.

Gaucher disease

Introduction	698	Treatment	704
Clinical abnormalities	699	References	705
Genetics and pathogenesis	703		

MAJOR PHENOTYPIC EXPRESSION

Type 1: Splenomegaly, pancytopenia, hepatomegaly, bony pain, fractures, avascular necrosis.

Type 2: Acute neuronopathic: Early infantile onset, hypertonicity, seizures, trismus, retroflexion of the head; splenomegaly; hepatomegaly; rapid neurologic deterioration and death between one and 24 months.

Type 3: Subacute neuronopathic: Splenomegaly, hepatomegaly; childhood onset of neurologic manifestations – ataxia, spastic paraparesis, seizures, ophthalmoplegia; death in childhood or adulthood if untreated.

All types: Accumulation glucocerebroside (glycosylceramide) and defective activity of lysosomal acid β -glucosidase.

INTRODUCTION

Gaucher disease is the most common of the lysosomal storage diseases. It was first described in 1882 by Gaucher [1], then a French medical student. He identified the pathognomonic cells, which are now known as Gaucher cells, in a 32-year-old woman with massive enlargement of the spleen. The eponym Gaucher disease was first employed by Brill in 1905 [2]. This phenotype, now referred to as type 1, was recognized in the 1950s to be common in Ashkenazi Jews [3, 4]. Two other types of disease are known (Table 93.1). The acute neuronopathic early infantile, type 2, disease was described in 1927 [5]. In 1959, a type 3, subacute neuronopathic disease was described in an isolated population in northern Sweden [6]; this slowly progressive neurologic disease is referred to as the Norrbottnian form, after the place of origin of the initial patients. Actually, each of the forms of the disease is panethnic.

Recognition of Gaucher disease as a reticuloendothelial storage disease was as early as 1907 [7], and in 1924 the stored material was identified as lipid and characterized as a cerebroside [8, 9]. Identification of the sugar in this cerebroside as glucose was reported by Aghion in 1934 in his thesis for the doctorate of philosophy (Figure 93.1) [10]. The molecular defect in glucocerebrosidase (Figure 93.2) was described in 1965, independently by Brady and

colleagues [11], and by Patrick [12]. The defective enzyme is a lysosomal acid β -glucosidase, active in catalyzing the release of glucose from a number of substrates in addition to glucosylceramide. There is an activator of the enzyme, saposin C, which has a low molecular weight [13]. The gene for β -glucosidase is located on chromosome 1q21 [14]. The cDNA has been cloned and a number and variety of mutations have been identified [15–17]. The type 1 disease provides an interesting therapeutic model because enzyme replacement therapy has been quite successful [18]. Bone marrow transplantation may be curative.

Table 93.1 Clinical presentations of Gaucher disease

	Type 1	Type 2	Type 3
Onset	Infants child/adulthood	3–6 months	Childhood
Neurodegeneration	Absent	++++	++ → ++++
Survival	6–80+ years	<2 Years	2nd to 4th decade
Splenomegaly	++++	++	++
Hepatomegaly	++	+	+
Fractures – bone crises	+	–	+
Ethnic predilection	Ashkenazi	Panethnic	Norrbottnian Swedish

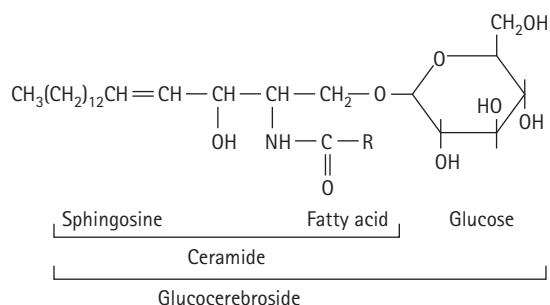


Figure 93.1 Structure of glucocerebroside, the Gaucher lipid.

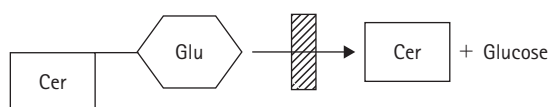


Figure 93.2 β -Glucocerebrosidase, the site of the defect in Gaucher disease.

CLINICAL ABNORMALITIES

Type 1

There is considerable heterogeneity of clinical expression. As many as 25 percent of affected individuals may be asymptomatic or have splenomegaly discovered incidental to an examination well into adult life, even into the eighth and ninth decades [19–22]. Severely affected individuals with type 1 disease may die in the first or second decade. The spleen may be massively enlarged.

The initial clinical manifestation is usually painless splenomegaly [23, 24]. The spleen may be massively enlarged (Figures 93.3 and 93.4). It may be so large as to interfere with the intake of food into the stomach or to cause dispareunia. Splenic infarcts may occur. A large infarction may produce the picture of an acute abdomen, along with hyperuricemia. Radionuclide scans may be helpful in the diagnosis of the splenic infarcts.

The liver is also enlarged (Figures 93.4, 93.5, 93.6, and 93.7), usually less than the spleen, but it may be as large or larger than the spleen (Figure 93.4) and it may be particularly prominent in splenectomized patients (Figures 93.5, 93.6, and 93.7). The large liver is usually not associated with liver disease, but there may be elevated transaminases, cirrhosis, esophageal varices, or hepatic failure [25–27]. Hepatic infarction may present as an acute abdominal catastrophe with a Budd-Chiari syndrome. One patient we saw was left with a large palpable notch in the center of the hepatic outline.

Thrombocytopenia is a common hematological manifestation of Gaucher disease [26]. It may be accompanied by leukopenia and anemia, the full picture



Figure 93.3 FTH: A two-year-old Saudi patient with Gaucher disease. Abdominal distention had been progressive and associated with weakness and failure to thrive. The liver was palpable 12 cm below the right costal margin. The spleen had been removed. β -Glucosidase activity of fibroblasts was 9 mmol/mg per hour or 5 percent of control.



Figure 93.4 ZYAA: A three-year-old girl with Gaucher disease. The abdomen was enormous and the patient emaciated. The liver was palpated at 20 cm and the spleen at 17 cm below the costal margins. Hemoglobin was 6 g/dL and platelet count $45 \times 10^3/\text{mm}^3$. β -Glucosidase activity was 1 mmol/hour per mg cells or 6 percent of control.



Figure 93.5 HFB: A four-year-old Saudi boy with Gaucher disease. The spleen had been removed, but the abdomen was distended by the enormous liver. In addition, he was thin, short, and wasted. Enzyme activity was 12 percent of control.



Figure 93.7 SSH: A three-year-old boy with Gaucher disease. The abdominal enlargement caused by the hepatomegaly is evident even with the patient fully clothed. β -Glucosidase activity was 5 percent of control. He had a splenectomy and had recently had successful bone marrow transplantation.



Figure 93.6 HFB: Lateral view highlights the abdominal distention. Parents were first cousins. IQ was 93. Treatment was initiated with ceredase (glucocerebrosidase).

of hypersplenism, and resolves with splenectomy [28]. Late hematological dysfunction in splenectomized patients may result from replacement of normal marrow with Gaucher cells. There may be bleeding, petechiae, and easy bruising.

Skeletal manifestations may be the chief or only complaint in some patients. Most patients have some bony abnormality. In many patients, they take the form of acute crises of pain, tenderness, redness, and swelling, mimicking acute osteomyelitis or the thrombotic crises of sickle cell disease.

There may be fever, leukocytosis, and elevation of the erythrocyte sedimentation rate. The diagnosis is best confirmed by technetium radionuclide scan [29]. Pyogenic osteomyelitis is rare in Gaucher disease and usually follows some invasive procedure on bone; therefore, surgical diagnostic procedures are not recommended for crises [30].

Areas of focal destruction of bone, osteonecrosis, or avascular necrosis occur in the absence of acute crises, especially in the area of the femoral head [31]. A child with hip pain may be thought to have Legg-Calve-Perthes disease [32]. Pathologic fracture is common. Some degree of osteoporosis is the rule in this disease. Compression fracture of vertebral bodies is a common complication [31, 33–35], and there may be radicular or spinal cord compression or kyphoscoliotic deformity. Roentgenograms reveal osteoporosis of the spine and compression fractures



Figure 93.8 Roentgenogram of the lower extremities of a patient with Gaucher disease illustrates the osteoporosis and enlargement of the width particularly of the femur leading to an Erlenmeyer flask appearance.

[36]. Magnetic resonance imaging (MRI) is more effective than conventional radiography or computed tomography (CT) scanning in evaluating the spine and effects on the cord and also in assessing areas of avascular necrosis [37]. The most common skeletal feature of Gaucher disease is the loss of modeling and increased width that leads to the Erlenmeyer flask appearance of the femur (Figure 93.8) [38]. In addition, areas of severe loss of bone density may alternate with areas of osteosclerosis and focal infarctions.

Growth and development may be altered drastically in Gaucher disease (Figure 93.9). Pubertal development may also be delayed. In addition to the effects of anemia, splenic enlargement, and chronic disease, resting energy expenditure is increased about 44 percent [39].

Pulmonary infiltration may also occur (Figure 93.10) and may lead to pulmonary failure [40]. There may also be right to left intrapulmonary shunting of blood. Fingers may be clubbed. The skin may show yellow or brown pigmentation and a propensity to tan [41]. Patients with Gaucher disease appear to be susceptible

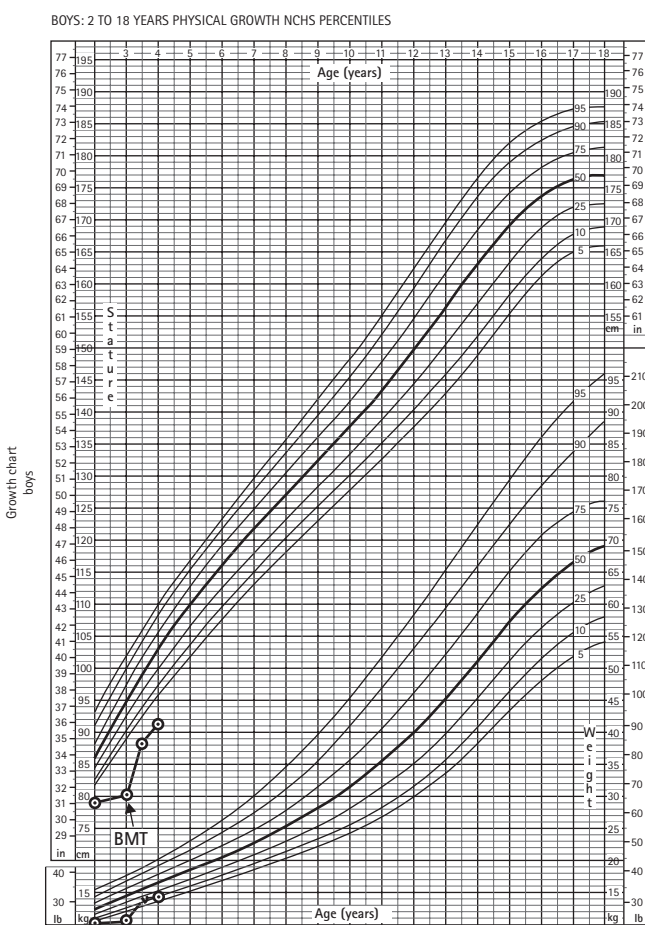


Figure 93.9 SSH: Acceleration in growth in height and weight following bone marrow transplantation. Adapted with permission from Hamill PV, Drizd TA, Johnson CL et al. Physical growth: National Center for Health Statistics percentiles. *Am J Clin Nutr* 1979; 32: 607–29.

to cancer, especially lymphoproliferative diseases [42], but there is considerable controversy as to whether there is statistically increased risk [43]. The nervous system is by definition spared in type 1 disease, but a number of secondary complications do affect the nervous system in addition to the complications of vertebral disease. These include cerebral fat emboli secondary to skeletal disease, neuropathy and hematomyelia secondary to bleeding [42]; a case was reported with lumbosacral cauda equina syndrome secondary to an intrathecal sacral cyst, apparently the result of subdural hemorrhage [44].

A few patients with type 1 Gaucher disease have been found to develop parkinsonian symptoms [45], with onset typically in the fourth decade and a limited responsiveness to therapy. There has not been any association with genotype, but in several cases there was a family history of parkinsonism [46]. In a study of tissue from patients with parkinsonism, alterations in glucocerebrosidase were found in 12 out of 57 individuals [47]. Overall, although the association between parkinsonism and Gaucher disease



Figure 93.10 FTH: Involvement of the lung was documented by biopsy. The patient was oxygen-dependent. Roentgenogram revealed virtually completely opaque lung fields with air bronchograms.

remains to be explained, it appears that deficiency of glucocerebrosidase confers predisposition to development of parkinsonism.

Type 2

Infants with acute neuronopathic Gaucher disease appear normal at birth, although splenomegaly may be found in the first three months, and they usually develop some early milestones. Early evidence of neurologic disease may be unusual irritability, a lack of alertness, apparent weakness in holding up the head, oculomotor apraxia, or a fixed strabismus [24, 48]. Neurodegenerative disease appears in six months and proceeds rapidly to a classic picture of spasticity and opisthotonus, with trismus, strabismus, and hyperextension of the neck [48]. There may be seizures, bulbar signs, or involuntary choreoathetoid movements. Visual fixation may be absent, as well as visual evoked response (VER). Atrophy may be evident on the MRI of the brain. Death usually results from apnea, aspiration pneumonia, or respiratory failure at an average age of 18 months. Autopsy reveals neuronal degeneration and neuronophagia.

A subtype of infants with this type of Gaucher disease has a rapid fulminant neonatal onset course [48, 49]. Death may occur as early as two months of age. This disorder is reminiscent of the disease in mice homozygous for a null glucocerebrosidase allele who die within 24 hours [50]. Infants with Gaucher disease may present with lamellar

ichthyosis, which may take the form of the collodion baby [51–53]. Presentation of Gaucher disease with non-immune hydrops fetalis may represent the same process [54]. Perinatal-lethal Gaucher disease with hydrops, ichthyosis, and fetal akinesia sequence has been associated with particularly severe mutations [55].

Type 3

In the classic Norrbottnian form of disease [56, 57], the patient's early manifestations may lead to a diagnosis of type 1 Gaucher disease. The neurologic keynote finding is myoclonus of cerebral origin [58, 59]. Multifocal jerky movements are widespread in the muscles and occur at rest or with movement. The electroencephalogram (EEG) may reveal spike discharges [60]. With time, there are generalized grand mal seizures [61, 62]. Careful examination of the eyes discloses deficits in saccadic velocities, progressive with time to paralysis of lateral gaze [63, 64]. There may be slow upward looping of the eyes [65]. As the disease progresses, ataxia and spasticity appear. There may be dementia.

A group of patients in whom paralysis of horizontal supranuclear gaze is the major neurologic sign have been considered as a subgroup of type 3 [48, 64]. They may have mild cognitive impairment [66]. These patients often have aggressive systemic manifestations. Some authors have subdivided type 3 patients into groups, for example designating those with progressive myoclonic epilepsy and dementia, and a poorer prognosis, as type 3a [64], but there are clearly unidentified genetic modifiers which determine prognosis [67]. When untreated, death in type 3 patients characteristically occurs in childhood or adolescence from pulmonary or hepatic disease, and in those with progressive myoclonic epilepsy, neurologic deterioration may cause death in adulthood despite treatment.

Diagnosis

The diagnosis is often first made clinically by the recognition of Gaucher cells (Figures 93.11 and 93.12) in a bone marrow aspirate or biopsied tissue. These cells are large lipid-laden macrophages with foamy cytoplasm. The fibrillary pattern is quite different from that of Niemann-Pick cells. Electron microscopy reveals tubular structures. These cells are widely dispersed in tissues. The diagnosis may be suspected by the presence of elevated activity of acid phosphatase in plasma [68], a high level of ferritin [69], angiotensin-converting enzyme [70], and particularly chitotriosidase, which is induced in activated macrophages, and is very significantly elevated in most cases of Gaucher disease [70].

Definitive diagnosis requires the assay of acid β -glucosidase. This can be done in leukocytes or cultured fibroblasts [71–73]. Enzyme assay is not useful in distinguishing the various types of Gaucher disease.

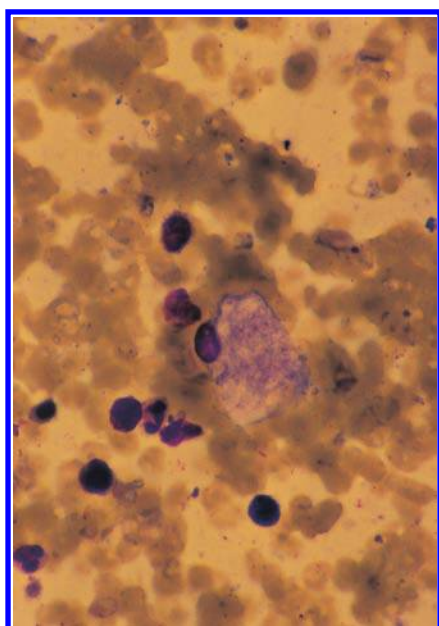


Figure 93.11 Gaucher cells. At this power, the size relative to leukocytes and erythrocytes is evident.

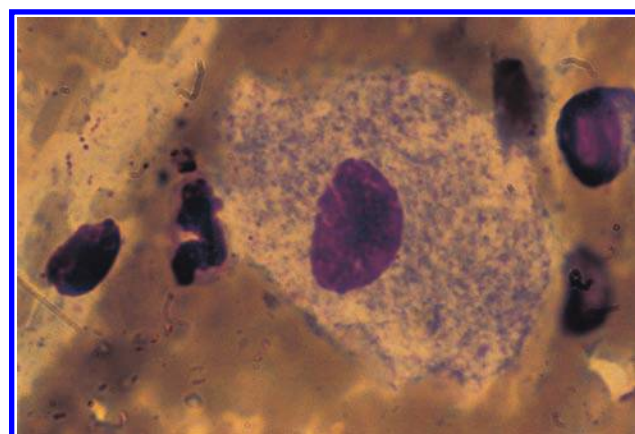


Figure 93.12 Gaucher cells illustrating the foamy cytoplasm.

GENETICS AND PATHOGENESIS

Gaucher disease is inherited in an autosomal recessive fashion. Most patients are of type 1, and this is most common in Ashkenazi Jews, in whom the incidence has been estimated at between one in 640 [24] and one in 855 [74]. This is the most prevalent genetic disorder in that population.

The gene for acid β -glucosidase has been cloned and linked to chromosome 1q21 [14, 17, 75]. It contains 11 exons and is approximately 7 kb in length. The cDNA is about 2.5 kb [16, 76]. More than 200 mutations have been defined in patients with Gaucher disease [77, 78]. Four are common enough to account for over 96 percent of the Ashkenazi Jewish patients (Table 93.2) [79, 80]. The A to G transition at position 1226 which causes an asparagine to serine substitution at amino acid 370 (N370S) [80] is the major cause of the disease in this population. The next most frequent is the frameshift mutation at 84GG [81], a mutation which leads to no enzyme protein. The

point mutation which changes a leucine to proline at 444 (L444P) and the splice junction mutation at IVS2(+1)G>A account for most of the rest of mutations in this population [17, 28].

Heterozygote detection is sometimes possible by enzyme analysis, but it is not reliable because of overlap with the normal population. In Jewish populations, heterozygote detection may be carried out by genotyping. This is less useful in non-Jewish populations; about 25 percent of these patients carry the N370S mutation and 35 percent the L444P mutation, and the rest are unidentified or rare.

Some correlations of phenotype with genotype have emerged from increasing information on the nature of mutation in Gaucher disease. The N370S mutation appears relatively conservative and a majority of patients who carry this mutation have relatively mild disease. Homozygosity for this mutation excludes neuronopathic disease, but even a single 370 allele in a compound leads to an absence of neurologic disease [76]; compound heterozygotes tend to have more severe somatic disease than N370S homozygotes. The Norrbottnian population of Gaucher patients in northern Sweden are homozygous for the L444P mutation and have type 3 disease of variable severity [82]. The L444P mutation is also found in other populations, and though it is associated with severe somatic symptoms and generally with neurologic

Table 93.2 Common mutations in Gaucher disease

cDNA No.	Amino acid No.	Nucleotide substitution	Amino acid substitution	Type of mutation
1226	370	A \rightarrow G	Asn Ser	Point mutation
84		G \rightarrow GG		Frameshift insertion
1448	444	T \rightarrow C	Leu Pro	Point mutation
IVS2 + 1		G \rightarrow A		Splice junction mutation

symptoms, in some patients neuropathic symptoms may be absent [64]. The 84GG frameshift leads to severe disease and no enzyme activity, as does the IVS2(+1)G>A mutation in intron 2 [28, 79, 83].

An index of complexity, and the power of molecular techniques to unravel it, is a family [84] in which two children died of type 2 disease, and a son had relatively indolent type 1 disease. The mother and son had similarly low levels of β -glucosidase, while the father's level was consistent with heterozygosity. Molecular analysis revealed the mother, asymptomatic at 62 years, and the son to be S370N/L444P compounds. The father was heterozygous L444P/normal. It was assumed that the infants who died by one year of age had inherited two L444P alleles.

The consequence of defective activity of acid β -glucosidase is the accumulation of glycosylceramide (Figure 93.1). In ceramide, there is a long chain fatty acid amide linkage at the carbon 2 of sphingosine. Glycosphingolipids with longer oligosaccharide moieties are successively degraded (Chapter 91) to glycosylceramide. The amounts of this compound stored in Gaucher disease are enormous. Deacylated glycosylsphingosine has also been found in tissues of patients [85]. It is thought that the accumulated compounds are toxic to certain tissues.

TREATMENT

Among the earliest approaches to provide a source of active enzyme in Gaucher disease was the use of bone marrow transplantation (Figure 93.9) [86]. The procedure may be essentially curative in type 1 disease, but there is a considerable risk of mortality from the procedure. It certainly raises the possibility that gene therapy in which the normal gene is introduced into the patient's hematopoietic cells will one day be an option.

Meanwhile, enzyme replacement therapy has become a major advance in the management of this disease. Gaucher disease was the first lysosomal storage disease for which this approach became available. The major breakthrough in permitting successful therapy was the recognition that lipid-laden macrophages have a mannose receptor [87]; modifying the glycoprotein glucocerebrosidase to expose a terminal mannose permits the enzyme to attach to and be incorporated into the macrophage [48, 88]. A modified form of the enzyme purified from human placenta was approved for treatment in 1991 under the name Ceredase (alglucerase), and then in 1994 a form of human glucocerebrosidase produced in cultured Chinese hamster ovary cells was approved under the name Cerezyme (imiglucerase). Another form, produced in human fibroblasts, was approved in 2010 under the name VPRIV (velaglucerase).

Clinical responses to enzyme replacement therapy have been clearly evident in hematological (anemia and thrombocytopenia), visceral, and even (with longer courses of treatment) bony disease. Decrease in organ

size is evident within six months. Bone pains diminish or disappear and, at least in children, roentgenograms of the bones have improved after years of treatment. Growth and development regularly improve. The treatment is expensive. There is still some controversy as to dose. The dose generally employed is 60 U/kg every 2 weeks [48], but 30 U/kg every 2 weeks may also be effective [89]. Success had also been reported with 2.3 U/kg three times a week [90]. There is logic in using small frequent infusions considering that the macrophage receptors constitute a low capacity, high affinity system, but practical considerations of intravenous access and demands on the patient's time have made the larger, less frequent dosing much more popular. It is possible that maintenance requirements for enzyme will be lower after the initial removal of accumulated glycolipid [91], but there is a risk of relapse in some patients.

Another approach, called substrate reduction therapy, is possible with an inhibitor of ceramide glucosyltransferase, and the agent N-butyldeoxynojirimycin was approved in 2004 (miglustat) for adult patients with Gaucher disease for whom intravenous enzyme replacement is not practical [92, 93]. Studies are underway using a more specific glucosylceramide synthase inhibitor, eliglustat tartrate [94]. An alternative approach is to use pharmacologic chaperones to stabilize products of missense mutations, and there are efforts to develop such agents, including isofagomine [95].

It is generally agreed that enzyme replacement is not effective in type 2 disease, while in the type 3 disease, systemic improvement is accompanied by no change in cerebral manifestations [96]. At present, only supportive measures are available for type 2 disease. Piracetam may be helpful in the management of myoclonus [97].

A variety of modalities should be used to monitor the progress of therapy in Gaucher disease. Dual-energy X-ray absorptiometry (DXA) is sensitive to changes in bone mineral density and generalized osteopenia, but is insensitive to local changes and cannot reliably predict fracture risk [98]. It is recommended that conventional MRI be used, and methods using chemical shift reflect the differences in the resonant frequencies of fat and water in bone marrow and can therefore detect the reduction in the fat fraction of bone marrow that occurs with infiltration in Gaucher disease. The preferred method is quantitative chemical shift imaging [99], but that is not widely available. Other approaches for semi-quantitative estimation of bone marrow burden of disease use a scoring system [100]. The most sensitive biomarker which correlates with the disease activity is chitotriosidase [69], but approximately 6 percent of the general population has no activity, due to a panethnic inactivating 24-bp duplication [101]. Another marker, CCL18 (also known as PARC/MIP-4/DC-CK1), is elevated ten- to 50-fold in symptomatic Gaucher disease patients [102].

A variety of supportive measures may be rendered unnecessary by the early use of enzyme replacement.

There is in general no longer a place for splenectomy, but it might be considered in a patient with extensive thrombocytopenia or cardiopulmonary symptoms from a massive spleen. Hip replacement is the preferred modality for avascular necrosis. Replacement of other joints may be necessary. The avoidance of injury to bone, especially in sports is prudent. The frequency and severity of crises of bone pain and fractures were reported to improve in patients treated with biphosphonates [103], which are analogs of pyrophosphate that bind to hydroxyapatite, inhibiting resorption. A placebo-controlled trial of alendronate showed significant benefit as an adjuvant to enzyme replacement, with improvement in bone density and bone mineral content within 18 months [104].

REFERENCES

- Gaucher PCE. De l'épithélioma primitif de la rate, hypertrophie idiopathique de la rate sans leucémie. Thesis, Paris, 1882.
- Brill NE, Mandelbaum FS, Libman E. Primary splenomegaly-Gaucher type. Report on one of few cases occurring in a single generation of one family. *Am J Med Sci* 1905; **129**: 491.
- Fried K. Gaucher's disease among the Jews of Israel. Proceedings of the Fourth Meeting of the Israel Genetics Circle. *Bull Res Counc Isr* 1958; **7B**: 213.
- Fried K. Population study of chronic Gaucher's disease. *Isr J Med Sci* 1973; **9**: 1396.
- Oberling C, Woringer P. La maladie de Gaucher chez la nourrisson. *Rev Fr Pédiatr* 1927; **3**: 475.
- Hillborg PO. Morbus Gaucher: Norbotten. *Nord Med* 1959; **61**: 303.
- Marchand F. Über sogenannte idiopathische Splenomegalie (Typus Gaucher). *Munch Med Wochenschr* 1907; **54**: 1102.
- Epstein E. Beitrag zur Chemie der Gaucherschen Krankheit. *Hoppe-Seyler's Z Physiol Chem* 1924; **271**: 211.
- Lieb H. Der Zucker im Cerebrosid der Milz bei der Gaucher Krankheit. *Hoppe-Seyler's Z Physiol Chem* 1924; **271**: 211.
- Aghion A. La maladie de Gaucher dans l'enfance. Thesis, Paris, 1934.
- Brady RO, Kanfer JN, Shapiro D. Metabolism of glucocerebrosides. II. Evidence of an enzymatic deficiency in Gaucher's disease. *Biochem Biophys Res Commun* 1965; **18**: 22.
- Patrick AD. A deficiency of glucocerebrosidase in Gaucher's disease. *Biochem J* 1965; **97**: 17c.
- Ho MW, O'Brien JS. Gaucher's disease: deficiency of 'acid'-glucosidase and reconstitution of enzyme activity *in vitro*. *Proc Natl Acad Sci USA* 1971; **68**: 2810.
- Barneveld RA, Keijzer W, Tegelaers FP *et al*. Assignment of the gene coding for human beta-glucocerebrosidase to the region q21-q31 of chromosome 1 using monoclonal antibodies. *Hum Genet* 1983; **64**: 227.
- Ginns EI, Choudary PV, Martin BM *et al*. Isolation of cDNA clones for human beta-glucocerebrosidase using the lambda gt11 expression system. *Biochem Biophys Res Commun* 1984; **123**: 574.
- Sorge J, West C, Westwood B, Beutler E. Molecular cloning and nucleotide sequence of human glucocerebrosidase cDNA. *Proc Natl Acad Sci USA* 1985; **82**: 7289.
- Tsuji S, Choudary PV, Martin BM *et al*. A mutation in the human glucocerebrosidase gene in neuronopathic Gaucher's disease. *N Engl J Med* 1987; **316**: 570.
- Barton NW, Brady RO, Dambrosia JM *et al*. Replacement therapy for inherited enzyme deficiency - macrophage-targeted glucocerebrosidase for Gaucher's disease. *N Engl J Med* 1991; **324**: 1464.
- Berrebi A, Wishnitzer R, der-Walde U. Gaucher's disease: unexpected diagnosis in three patients over seventy years old. *Nouv Rev Fr Hematol* 1984; **26**: 201.
- Brinn L, Glabman S. Gaucher's disease without splenomegaly. Oldest patient on record, with review. *NY State J Med* 1962; **62**: 2346.
- Chang-Lo M, Yam LT, Rubenstein AI. Gaucher's disease. Review of the literature and report of twelve new cases. *Am J Med Sci* 1967; **254**: 303.
- Beutler E. Gaucher's disease in an asymptomatic 72-year-old. *J Am Med Assoc* 1977; **237**: 2529.
- Matoth Y, Fried K. Chronic Gaucher's disease; clinical observations on 34 patients. *Isr J Med Sci* 1965; **1**: 521.
- Kolodny EH, Ullman MD, Mankin HJ *et al*. Phenotypic manifestations of Gaucher disease: clinical features in 48 biochemically verified type 1 patients and comment on type 2 patients. *Prog Clin Biol Res* 1982; **95**: 33.
- Morrison AN, Lane M. Gaucher's disease with ascites: a case report with autopsy findings. *Ann Intern Med* 1955; **42**: 1321.
- Javett SN, Kew MC, Liknaitsky D. Gaucher's disease with portal hypertension: case report. *J Pediatr* 1966; **68**: 810.
- James SP, Stromeyer FW, Stowens DW, Barranger JA. Gaucher disease: hepatic abnormalities in 25 patients. *Prog Clin Biol Res* 1982; **95**: 131.
- Beutler E, Gelbart T, Kuhl W *et al*. Mutations in Jewish patients with Gaucher disease. *Blood* 1992; **79**: 1662.
- Katz K, Mechlis-Frish S, Cohen IJ *et al*. Bone scans in the diagnosis of bone crisis in patients who have Gaucher disease. *J Bone Joint Surg Am* 1991; **73**: 513.
- Lachiewicz PF. Gaucher's disease. *Orthop Clin North Am* 1984; **15**: 765.
- Amstutz HC, Carey EJ. Skeletal manifestations and treatment of Gaucher's disease: review of twenty cases. *J Bone Joint Surg Am* 1966; **48-A**: 670.
- Kenet G, Hayek S, Mor M *et al*. The 1226G (N370S) Gaucher mutation among patients with Legg-Calve-Perthes disease. *Blood Cells Mol Dis* 2003; **31**: 72.
- Goldblatt J, Sacks S, Beighton P. The orthopedic aspects of Gaucher disease. *Clin Orthop* 1978; **137**: 208.
- Hermann G, Goldblatt J, Desnick RJ. Kummell disease: delayed collapse of the traumatised spine in a patient with Gaucher type 1 disease. *Br J Radiol* 1984; **57**: 833.
- Raynor RB. Spinal-cord compression secondary to Gaucher's disease. Case report. *J Neurosurg* 1962; **19**: 902.
- Katz K, Sabato S, Horev G *et al*. Spinal involvement in children and adolescents with Gaucher disease. *Spine* 1993; **18**: 332.

37. Rosenthal DI, Scott JA, Barranger J *et al.* Evaluation of Gaucher disease using magnetic resonance imaging. *J Bone Joint Surg Am* 1986; **68**: 802.
38. Mankin HJ, Doppelt SH, Rosenberg AE, Barranger JA. Metabolic bone disease in patients with Gaucher's disease. In: Aviali LV, Krane SM (eds). *Metabolic Bone Disease and Clinically Related Disorders*, 2nd edn. Philadelphia: WB Saunders, 1990: 730–52.
39. Barton DJ, Ludman MD, Benkov K *et al.* Resting energy expenditure in Gaucher's disease type 1: effect of Gaucher's cell burden on energy requirements. *Metabolism* 1989; **38**: 1238.
40. Schneider EL, Epstein CJ, Kaback MJ, Brandes D. Severe pulmonary involvement in adult Gaucher's disease. Report of three cases and review of the literature. *Am J Med* 1977; **63**: 475.
41. Goldblatt J, Beighton P. Cutaneous manifestations of Gaucher disease. *Br J Dermatol* 1984; **111**: 331.
42. Grewal RP, Doppelt SH, Thompson MA *et al.* Neurologic complications of nonneuronopathic Gaucher's disease. *Arch Neurol* 1991; **48**: 1271.
43. Choy FY, Campbell TN. Gaucher disease and cancer: concept and controversy. *Int J Cell Biol* 2011; **2011**: 150450.
44. Hamlat A, Saikali S, Lakehal M *et al.* Cauda equina syndrome due to an intra-dural sacral cyst in type-1 Gaucher disease. *Eur Spine J* 2004; **13**: 249.
45. Neudorfer O, Giladi N, Elstein D *et al.* Occurrence of Parkinson's syndrome in type I Gaucher disease. *Q J Med* 1996; **89**: 691.
46. Tayebi N, Walker J, Stubblefield B *et al.* Gaucher disease with parkinsonian manifestations: does glucocerebrosidase deficiency contribute to a vulnerability to parkinsonism? *Mol Genet Metab* 2003; **79**: 104.
47. Lwin A, Orvisky E, Goker-Alpan O *et al.* Glucocerebrosidase mutations in subjects with parkinsonism. *Mol Genet Metab* 2004; **81**: 70.
48. Sidransky E, Ginns EI. Clinical heterogeneity among patients with Gaucher's disease. *J Am Med Assoc* 1993; **269**: 1154.
49. Martin BM, Sidransky E, Ginns EI. Gaucher's disease: advances and challenges. *Adv Pediatr* 1989; **36**: 277.
50. Tybulewicz VL, Tremblay ML, LaMarca ME *et al.* Animal model of Gaucher's disease from targeted disruption of the mouse glucocerebrosidase gene. *Nature* 1992; **357**: 407.
51. Lui K, Commens C, Choong R, Jaworski R. Collodion babies with Gaucher's disease. *Arch Dis Child* 1988; **63**: 854.
52. Lipson AH, Rogers M, Berry A. Collodion babies with Gaucher's disease – a further case. *Arch Dis Child* 1991; **66**: 667.
53. Girgensohn H, Kellner H, Südhof H. Angeborener Morbus Gaucher bei Erythroblastose und Gefassverkalkung. *Klin Wochenschr* 1954; **32**: 57.
54. Sun CC, Panny S, Combs J, Gutberlett R. Hydrops fetalis associated with Gaucher disease. *Pathol Res Pract* 1984; **179**: 101.
55. Mignot C, Gelot A, Bessieres B *et al.* Perinatal-lethal Gaucher disease. *Am J Med Genet* 2003; **120A**: 338.
56. Erikson A. Gaucher disease – Norrbottnian type (III). Neuropaediatric and neurobiological aspects of clinical patterns and treatment. *Acta Paediatr Scand Suppl* 1986; **326**: 1.
57. Dreborg S, Erikson A, Hagberg B. Gaucher disease – Norrbottnian type I. General clinical description. *Eur J Pediatr* 1980; **133**: 107.
58. Miller JD, McCluer R, Kanfer JN. Gaucher's disease: neurologic disorder in adult siblings. *Ann Intern Med* 1973; **78**: 883.
59. King JO. Progressive myoclonic epilepsy due to Gaucher's disease in an adult. *J Neurol Neurosurg Psychiatry* 1975; **38**: 849.
60. Vinken PJ, Bruyn GW, Klawans HL (eds). *Handbook of Clinical Neurology*, vol. 5: Extrapyrmidal disorders. Amsterdam: Elsevier, 1986.
61. Tripp JH, BD Lake, E Young *et al.* Juvenile Gaucher's disease with horizontal gaze palsy in three siblings. *J Neurol Neurosurg Psychiatry* 1977; **40**: 470.
62. Grover WD, Tucker SH, Wenger DA. Clinical variation in two related children with neuronopathic Gaucher disease. *Ann Neurol* 1978; **3**: 281.
63. Sidransky E, Tsui S, Stubblefield BK *et al.* Gaucher patients with oculomotor abnormalities do not have a unique genotype. *Clin Genet* 1992; **41**: 1.
64. Patterson MC, Horowitz M, Abel RB *et al.* Isolated horizontal supranuclear gaze palsy as a marker of severe systemic involvement in Gaucher's disease. *Neurology* 1993; **43**: 1993.
65. Cogan DG, Chu FC, Reingold D, Barranger J. Ocular motor signs in some metabolic diseases. *Arch Ophthalmol* 1981; **99**: 1802.
66. Erikson A, Karlberg J, Skogman AL, Dreborg S. Gaucher disease (type III): intellectual profile. *Pediatr Neurol* 1987; **3**: 87.
67. Park JK, Orvisky E, Tayebi N *et al.* Myoclonic epilepsy in Gaucher disease: genotype–phenotype insights from a rare patient subgroup. *Pediatr Res* 2003; **53**: 387.
68. Lam KW, Li CY, Yam LT *et al.* Comparison of prostatic and nonprostatic acid phosphatase. *Ann NY Acad Sci* 1982; **390**: 1.
69. Zimran A, Kay A, Gelbart T *et al.* Gaucher disease. Clinical, laboratory, radiologic, and genetic features of 53 patients. *Medicine* 1992; **71**: 337.
70. Cabrera-Salazar MA, O'Rourke E, Henderson N *et al.* Correlation of surrogate markers of Gaucher disease. Implications for long-term follow up of enzyme replacement therapy. *Clin Chim Acta* 2004; **344**: 101.
71. Beutler E, Kuhl W. The diagnosis of the adult type of Gaucher's disease and its carrier state by demonstration of deficiency of beta-glucosidase activity in peripheral blood leukocytes. *J Lab Clin Med* 1970; **76**: 747.
72. Beutler E, Kuhl W, Trinidad F *et al.* Beta-glucosidase activity in fibroblasts from homozygotes and heterozygotes for Gaucher's disease. *Am J Hum Genet* 1971; **23**: 62.
73. Ho MW, Seck J, Schmidt D *et al.* Adult Gaucher's disease: kindred studies and demonstration of a deficiency of acid-glucosidase in cultured fibroblasts. *Am J Hum Genet* 1972; **24**: 37.
74. Beutler E, Nguyen NJ, Henneberger MW *et al.* Gaucher disease: gene frequencies in the Ashkenazi Jewish population. *Am J Hum Genet* 1993; **52**: 85.
75. Shafit-Zagardo B, Devine EA, Smith M *et al.* Assignment of the gene for acid beta-glucosidase to human chromosome 1. *Am J Hum Genet* 1981; **33**: 564.

76. Tsuji S, Choudary PV, Martin BM *et al*. Nucleotide sequence of cDNA containing the complete coding sequence for human lysosomal glucocerebrosidase. *J Biol Chem* 1986; **261**: 50.
77. Brady RO, Barton NW, Grabowski GA. The role of neurogenetics in Gaucher disease. *Arch Neurol* 1993; **50**: 1212.
78. Zhao H, Keddache M, Bailey L *et al*. Gaucher's disease: identification of novel mutant alleles and genotype-phenotype relationships. *Clin Genet* 2003; **64**: 57.
79. Beutler E. Gaucher disease: new molecular approaches to diagnosis and treatment. *Science* 1992; **256**: 794.
80. Strom CM, Crossley B, Redman JB *et al*. Molecular screening for diseases frequent in Ashkenazi Jews: lessons learned from more than 100,000 tests performed in a commercial laboratory. *Genet Med* 2004; **6**: 145.
81. Tsuji S, Martin BM, Barranger JA *et al*. Genetic heterogeneity in type 1 Gaucher disease: multiple genotypes in Ashkenazic and non-Ashkenazic individuals. *Proc Natl Acad Sci USA* 1988; **85**: 2349.
82. Beutler E, Gelbart T, Kuhl W *et al*. Identification of the second common Jewish Gaucher disease mutation makes possible population-based screening for the heterozygous state. *Proc Natl Acad Sci USA* 1991; **88**: 10544.
83. Dahl N, Lagerstrom M, Erikson A, Pettersson U. Gaucher disease type III (Norrbotnian type) is caused by a single mutation in exon 10 of the glucocerebrosidase gene. *Am J Hum Genet* 1990; **47**: 275.
84. Shahinfar M, Wenger DA. Adult and infantile Gaucher disease in one family: mutational studies and clinical update. *J Pediatr* 1994; **125**: 919.
85. Nilsson O, Svennerholm L. Accumulation of glucosylceramide and glucosylsphingosine (psychosine) in cerebrum and cerebellum in infantile and juvenile Gaucher disease. *J Neurochem* 1982; **39**: 709.
86. Hobbs JR. Experience with bone marrow transplantation for inborn errors of metabolism. *Enzyme* 1987; **38**: 194.
87. Ashwell G, Morell AG. The role of surface carbohydrates in the hepatic recognition and transport of circulating glycoproteins. *Adv Enzymol Relat Areas Mol Biol* 1974; **41**: 99.
88. Barton NW, Furbish FS, Murray GJ *et al*. Therapeutic response to intravenous infusions of glucocerebrosidase in a patient with Gaucher disease. *Proc Natl Acad Sci USA* 1990; **87**: 1913.
89. Pastores GM, Sibille AR, Grabowski GA. Enzyme therapy in Gaucher disease type 1: dosage efficacy and adverse effects in 33 patients treated for 6 to 24 months. *Blood* 1993; **82**: 408.
90. Figueroa ML, Rosenbloom BE, Kay AC *et al*. A less costly regimen of alglucerase to treat Gaucher's disease. *N Engl J Med* 1992; **327**: 1632.
91. Kishnani PS, DiRocco M, Kaplan P *et al*. A randomized trial comparing the efficacy and safety of imiglucerase (Cerezyme) infusions every 4 weeks versus every 2 weeks in the maintenance therapy of adult patients with Gaucher disease type 1. *Mol Genet Metab* 2009; **96**: 164–70.
92. Cox TM, Aerts JM, Andria G *et al*. The role of the iminosugar N-butyldeoxynojirimycin (miglustat) in the management of type I (non-neuronopathic) Gaucher disease: a position statement. *J Inherit Metab Dis* 2003; **26**: 513.
93. Pastores GM, Barnett NL. Substrate reduction therapy: miglustat as a remedy for symptomatic patients with Gaucher disease type 1. *Expert Opin Investig Drugs* 2003; **12**: 273.
94. Lukina E, Watman N, Arreguin EA *et al*. A phase 2 study of eliglustat tartrate (Genz-112638), an oral substrate reduction therapy for Gaucher disease type 1. *Blood* 2010; **116**: 893–9.
95. Khanna R, Benjamin ER, Pellegrino L *et al*. The pharmacological chaperone isofagomine increases the activity of the Gaucher disease L444P mutant form of beta-glucosidase. *FEBS J* 2010; **277**: 1618–38.
96. Bembi B, Zanatta M, Carrozzi M *et al*. Enzyme replacement treatment in type 1 and type 3 Gaucher's disease. *Lancet* 1994; **344**: 1679.
97. Obeso JA, Artieda J, Rothwell JC *et al*. The treatment of severe action myoclonus. *Brain* 1989; **112**: 765.
98. Maas M, Poll LW, Terk MR. Imaging and quantifying skeletal involvement in Gaucher disease. *Br J Radiol* 2002; **75**(Suppl. 1): A13.
99. Johnson LA, Hoppel BE, Gerard EL *et al*. Quantitative chemical shift imaging of vertebral bone marrow in patients with Gaucher disease. *Radiology* 1992; **182**: 451.
100. Maas M, van Kuijk C, Stoker J *et al*. Quantification of bone involvement in Gaucher disease: MR imaging bone marrow burden score as an alternative to Dixon quantitative chemical shift MR imaging – initial experience. *Radiology* 2003; **229**: 554.
101. Boot RG, Renkema GH, Verhoek M *et al*. The human chitotriosidase gene. Nature of inherited enzyme deficiency. *J Biol Chem* 1998; **273**: 25680.
102. Boot RG, Verhoek M, de Fost M *et al*. Marked elevation of the chemokine CCL18/PARC in Gaucher disease: a novel surrogate marker for assessing therapeutic intervention. *Blood* 2004; **103**: 33.
103. Samuel R, Katz K, Papapoulos SE *et al*. Aminohydroxy propylidene bisphosphonate (APD) treatment improves the clinical skeletal manifestations of Gaucher's disease. *Pediatrics* 1994; **94**: 385.
104. Wenstrup RJ, Bailey L, Grabowski GA *et al*. Gaucher disease: alendronate disodium improves bone mineral density in adults receiving enzyme therapy. *Blood* 2004; **104**: 1253.

Niemann–Pick disease

Introduction	708	Treatment	715
Clinical abnormalities	709	References	715
Genetics and pathogenesis	712		

MAJOR PHENOTYPIC EXPRESSION

Type A: Hepatosplenomegaly, neurologic degeneration, failure to thrive, cherry red macular spot, foam cells in bone marrow, storage of sphingomyelin and deficiency of lysosomal acid sphingomyelinase.

Type B: Hepatosplenomegaly, pulmonary infiltration, foam cells, storage of sphingomyelin and deficiency of sphingomyelinase.

INTRODUCTION

The disease was first described by Niemann in 1914 [1] in an infant with hepatosplenomegaly who died at 18 months after progressive neurologic deterioration and was found to have large foam cells in the liver and spleen. Pick's contribution [2, 3] was to distinguish this disorder from Gaucher disease on the basis of the appearance of the foam cells. Phenotypic variation became apparent with additional reports [4, 5], especially of adults with hepatosplenomegaly, but no neurologic abnormality in what has come to be called type B [6]. In 1934, the stored lipid was identified by Klenk [7] as sphingomyelin (Figure 94.1). In 1966, Brady *et al.* [8] identified the deficiency of sphingomyelinase (EC 3.1.4.12) (Figure 94.2) as the cause

of Niemann–Pick disease. The deficiency was also readily documented in a type B patient [9].

The discovery of the enzyme permitted the categorization of other patients classified as Niemann–Pick disease who clearly did not have sphingomyelinase deficiency as their molecular etiology, such as type C (Chapter 95). The separation of type C is an important distinction, but now that the nature of mutation has begun to be defined, separation into types A and B begins to appear artificial. There are certainly some very distinct phenotypes among the deficiencies of sphingomyelinase; soon the genotype for each will be known. It is already clear that there are quite distinct genotypes. Type A disease is relatively rare except in Ashkenazi Jews [5, 10, 11]; in whom type B is quite rare. Various phenotypes of type B are found

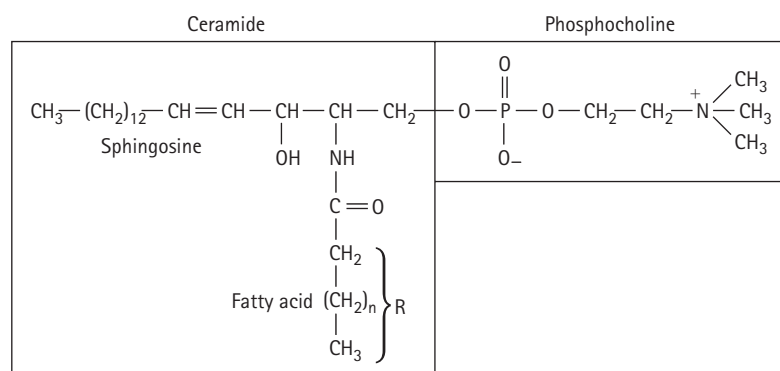


Figure 94.1 The structure of sphingomyelin.

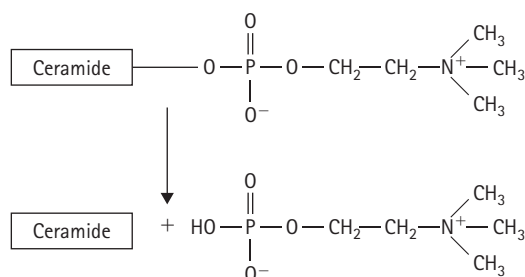


Figure 94.2 The sphingomyelinase reaction, the site of defect in Niemann-Pick disease.

commonly in Arabs, Turks, and Portuguese [12]. Somatic cell hybridization indicated clearly that types A and B represented allelic variation in a single gene [13] and that type C was different. Molecular studies were confirmatory.

The cDNA for sphingomyelinase has been cloned and sequenced [14, 15]. The gene has been mapped to chromosome 11 p15.1-p15.4 [16]. A number of mutations have been identified in both type A and type B patients [12, 17, 18]. Distinct mutations have been found in the ethnic groups in which Niemann-Pick disease is common.

CLINICAL ABNORMALITIES

Type A

The acute infantile form of Niemann-Pick disease usually presents first with massive enlargement of the liver and spleen (Figures 94.3, 94.4, and 94.5) [5, 6, 10, 19–21]. Some patients have neonatal edema and hydrops fetalis may occur [22]. The liver and spleen may be enlarged at birth, and storage of lipid has been documented in liver, brain, kidney, and placenta prior to birth [19, 23, 24]. Placental enlargement has been shown by ultrasound [25] and it is thought that storage in the placenta may lead to fetal loss [19]. The abdomen is protuberant. The liver seems always to be enlarged out of proportion to the spleen, in contrast to Gaucher disease (Chapter 93).

We have seen Niemann-Pick patients with hepatosplenomegaly whose history was that the spleen was not palpable early. Transaminases aspartate transaminase (AST) and alanine transaminase (ALT) are elevated, at least at times [26]. The alkaline phosphatase is also usually elevated. The cholesterol may be elevated in addition. There may be prolonged neonatal jaundice, and episodes of unexplained jaundice later. We have seen patients who presented in early infancy with acute jaundice, abnormal liver function tests, and hepatomegaly, suggesting a diagnosis of acute hepatocellular disease rather than a lipid storage disease. We have also seen a patient in whom two liver biopsies had been done in another institution and interpreted as fatty metamorphosis. At least one patient with Niemann-Pick disease was thought on



Figure 94.3 A patient with infantile Niemann-Pick disease. The hepatosplenomegaly is outlined.



Figure 94.4 Another patient with massive hepatosplenomegaly due to Niemann-Pick disease.

biopsy to have glycogenosis [27]. Jaundice is a common terminal finding and some patients have developed disseminated intravascular coagulopathy. There may be lymphadenopathy.

By six months, episodes of respiratory distress occur, which may require oxygen. Some episodes are clear-cut infections, such as bronchiolitis or pneumonia, but in others infection is not obvious. Some patients have had



Figure 94.5 Massive hepatosplenomegaly in an 11-month-old infant with Niemann–Pick disease. Abdominal distension was noted at birth. He had jaundice and acholic stools at six months, and repeated pulmonary infections.

noisy respirations and rhinorrhea from birth [28]. Chest film may reveal diffuse interstitial infiltrates in a reticular or finely nodular pattern [29]. Patients may also have unexplained fevers.

Failure to thrive is evident by eight to nine months of age. Weight gain stops, but increase in linear growth may not stop until 15–18 months of age, and so the patient looks increasingly cachectic. Anorexia may be complicated by vomiting, and there may be some diarrhea [30, 31] or constipation.

Neurologic involvement may be first evidenced in a failure to achieve milestones, such as sitting, but some have developed normally for six months [28], or as long as a year [6]. Progression of disease occurs with loss of milestones achieved. Patients may appear weak or hypotonic. Deep tendon reflexes are exaggerated. Neurologic degeneration is progressive to a rigid state with spasticity in which there appears to be no consciousness of the environment. Seizures are not common; the electroencephalograph (EEG) is usually normal. Cherry red or cherry black (dependent on the pigment of the patient) macular spots (Figure 94.6) are seen in about 50 percent of the patients. Sometimes, there is a sprinkled salt appearance around the macula, a gray granular appearance, the macular halo syndrome, or melting snow appearance [32–35]. The electroretinogram is abnormal.

Brownish-yellow discoloration may develop in the skin [36]. Xanthomas have been described, particularly on the face and arms [5, 10, 11]. Most patients develop osteoporosis. A hypochromic, microcytic anemia may be followed with



Figure 94.6 The cherry red macular degeneration in a ten-month-old child with Niemann–Pick disease.

time by thrombocytopenia or granulocytopenia. The terminal episode may be with asphyxia or pneumonia.

Type B

Type B patients represent quite a varied spectrum, from those diagnosed in infancy because of hepatomegaly or hepatosplenomegaly to those first detected in adulthood, as expected for a variety of different mutations. Nevertheless, the type A phenotype is much more common and accounts for about three-fourths of all patients. We suspect that among the type B patients there are a number of distinct phenotypes that are beginning to correlate with genotype.

What we think of as the classic type B patient is an adult or older adolescent who comes to attention because of splenomegaly found incidentally on physical examination (Figures 94.7 and 94.8) [37–39]. Some of these patients have had sea-blue histiocytes in marrow [37] or tissues [39] and this type has been called the Lewis variant [37]. Such patients may have elevation of the serum level of acid phosphatase. The King Faisal series now includes 35 patients. Pancytopenia may result from hypersplenism, and splenectomy may be required. Splenic rupture has been described [37]. Patients have been described in whom there were no neurological abnormalities well into adult life [5, 39–41]. This may be one phenotype.

Others with a relatively mild phenotype may have some neurologic features. Extrapyrarnidal signs were reported in one family [42]. Impaired mental development was reported in unrelated patients at nine and 18 years [43]. A number of patients have been reported with cerebellar ataxia [18, 42, 43]. Some of these may have been patients with Niemann–Pick type C disease. Patients have had cherry red spots or other grayish macular pigmentation about the macula, often with no other neurologic manifestation [32, 33, 39, 44–47]. Evidence of abnormal neural storage has been observed despite absence of neurologic abnormalities



Figure 94.7 A 39-year-old man with Lewis variant of Niemann-Pick disease. The spleen was palpable 6 cm below the costal margin. The liver was at 4 cm. Sphingomyelinase activity of fibroblasts was 18 percent of control.



Figure 94.8 Brownish pigment on the dorsal and lateral ankle correlated with a loss of vibratory sensation in the area.

[48]. Two sisters without impaired mental development had inclusion bodies in exons and Schwann cells of rectal biopsies, and vacuolated macrophages in the cerebrospinal fluid (CSF) [48].

Some others that have been included in type B have had quite severe, and early-onset disease. We think of this as the Saudi variant [49, 50]. Early symptoms are failure to thrive and abdominal distension. On examination, the spleen is huge (Figures 94.3, 94.4, and 94.5). The liver may be just as huge or even more so. They have been said not

to have neurodegenerative disease, but they all have cherry red macular spots (Figure 94.6). Furthermore, two patients who survived infancy and bone marrow transplantation went on to develop white matter changes in the central nervous system (CNS) and neurological manifestations. The facial appearance may develop similarities (Figure 94.9).

Patients are hypotonic and developmentally delayed. One patient at 20 months could sit, but could not crawl or stand [50]. She could speak two or three words. Cachexia is prominent early (Figures 94.10 and 94.11). Most of these patients die by three years of age. Terminal events include bleeding, anemia, and thrombocytopenia, often requiring daily transfusion of platelets, and hepatic failure. In a series of Saudi patients, some of whose pictures are shown in this chapter, five died between 18 and 36 months; one survivor to 4.5 years had had a bone marrow transplantation. Pulmonary infiltration is evident in roentgenograms as miliary nodular lesions [50]. Pulmonary function may be abnormal, and there may be complicating pneumonia. Liver function tests, alanine aminotransferase, and aspartate aminotransferase may be elevated, along with triglycerides. Abdominal ultrasound documents the hepatosplenomegaly.

Other patients may have hepatic or pulmonary disease. Liver disease, either biliary cirrhosis [51], or cirrhosis, may be life-threatening, and portal hypertension and ascites may develop [52]. This latter picture was reported in an otherwise adult-type disease [52]. Pulmonary disease has also been reported in adult-type disease [53]. In addition to the diffuse infiltration seen on roentgenograms, there may be exertional dyspnea and decreased pO_2 because of diminished diffusion. Bronchopneumonia may develop, and/or cor pulmonale [53].

In a series of type B patients in the United States, in whom sphingomyelinase deficiency and the mutation were documented, height and weight were usually low; 39 percent and 21 percent were below the fifth percentile for height and weight, and these correlated with large organ volumes [54]. Bone age was also behind 2.5 years.

Common pathological features

The pathognomonic feature of all patients with deficiency of sphingomyelinase is the foam cell (Figure 94.12). This large (20–90 μ) cell or macrophage is most commonly first detected in the bone marrow aspirate. As a reticuloendothelial cell, it is found widely in these patients' spleen, liver, lymph nodes, and lungs. In stained preparations, the cells have a foamy appearance that results from the stored material, which stains positively with stains for lipids. The lipid droplets are uniform in size, and the appearance has been called honeycomb-like or mulberry-like. The cytoplasm of these cells stains blue with Wright stain, which gives rise to the sea-blue histiocyte designation [37]. It is clear now that sea-blue histiocytes, once



Figure 94.9 Four Saudi infants (A–D) with Niemann–Pick disease illustrating some similarity of facial features. Patients tend to lose adipose tissue over the forehead and about the orbits; the nasal bridge is spared, giving the appearance of a crest of tissue.

thought to represent a distinct disease [55, 56] are seen in sphingomyelinase deficiency [39]. On electron microscopy (Figures 94.13 and 94.14), foam cells have small eccentric nuclei and membrane-bound lucent areas from which storage material has been dissolved. There may be granular material, whorls, or lamellae. There may be infiltration in the gastrointestinal tract, which might account for intestinal symptoms and failure to thrive, and diagnosis has been made by rectal biopsy. Storage is seen in neuronal cells and axons, and cerebral atrophy and neuroaxonal dystrophy are characteristic of type A disease.

GENETICS AND PATHOGENESIS

Niemann–Pick disease is transmitted as an autosomal recessive disease. The disease has been seen widely throughout the population of the world. The frequency of type A is much higher in Ashkenazi Jewish populations in which type B disease is rare.

The molecular defect is in the enzyme sphingomyelinase (Figure 94.2) [8, 9]. The enzyme was first purified from rat liver [57]. It cleaves the phosphocholine moiety from sphingomyelin. It is a lysosomal enzyme with

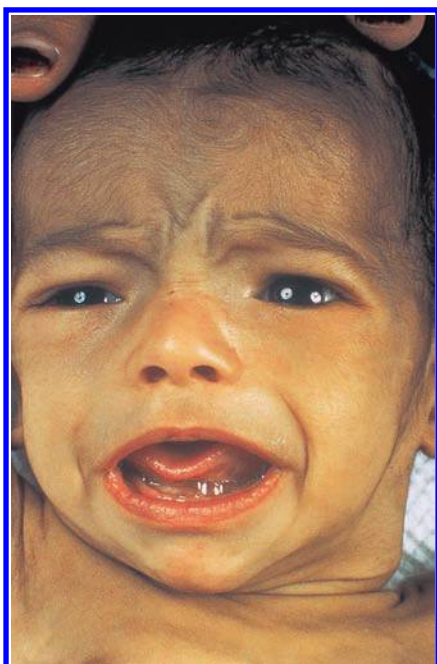


Figure 94.10 Another Saudi infant with Niemann-Pick disease, illustrating a more advanced degree of emaciation of the face. The eyes appear sunken.



Figure 94.11 This Saudi infant illustrates the extreme emaciation and the large abdomen resulting from organomegaly.

a pH optimum about 4.5 and a molecular weight of approximately 70 kDa [58, 59]. The cDNA predicts a protein monomer of 64 kDa; if the six potential glycosylations were filled the molecular weight would be 72–74 kDa. There are a number of sphingomyelinase

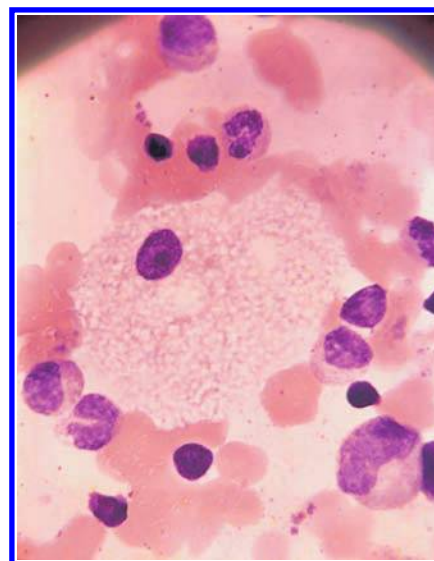


Figure 94.12 The Niemann-Pick foam cells of the bone marrow.

activator proteins (SAPs), but they do not appear to be required for enzymatic activity.

The defect has been demonstrated in tissues such as liver, kidney, and brain [60, 61], cultured fibroblasts [40, 62, 63], and leukocytes [64, 65]. Patients with type A and type B are defective in the same enzyme. Clinical diagnosis is generally based on the assay of leukocytes or fibroblasts. In general, in type A patients there is less than five percent of control activity, and often activity is undetectable. In type B disease activity is variable and may be higher, up to 10 percent of control [40, 59, 61], but it may also be zero in type B. Residual activity is not a reliable index of clinical severity.

In order to approximate physiological conditions more closely, a number of investigators have explored intact cell assays in which ^{14}C -labeled or fluorescent natural substrate was taken up and transported to lysosomes and then hydrolyzed [40, 63–65]. In these studies, substantial hydrolysis of sphingomyelin was demonstrable in type B cells, while very little occurred in those of type A.

Heterozygotes for types A and B disease generally have enzyme activity that is intermediate between those of homozygotes and normal [13, 66]. In fact, some heterozygotes have had splenomegaly or foam cells in the marrow. On the other hand, heterozygote detection may not be reliably excluded, because of overlap with the normal range.

Prenatal diagnosis has been undertaken by enzyme analysis in cultured amniocytes [40] and chorionic villus material [67], and a number of affected fetuses have been detected [68]. The intact cell assay with labeled sphingomyelin has also been effectively employed with cultured amniocytes [40].

The nature of the enzyme defect leads to the accumulation of sphingomyelin in tissues. This phospholipid is

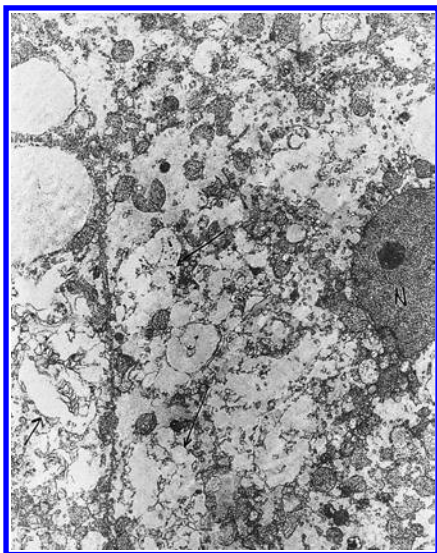


Figure 94.13 Electron microscopy of biopsied liver of an infant with classic infantile Niemann–Pick disease and deficiency of sphingomyelinase. Hepatocytes were large and pale. Electron microscopy, with the nucleus on the right, illustrates many irregular rounded membrane-bound lucent areas. These are considered to have contained lipid, which was extracted ($\times 10,000$).

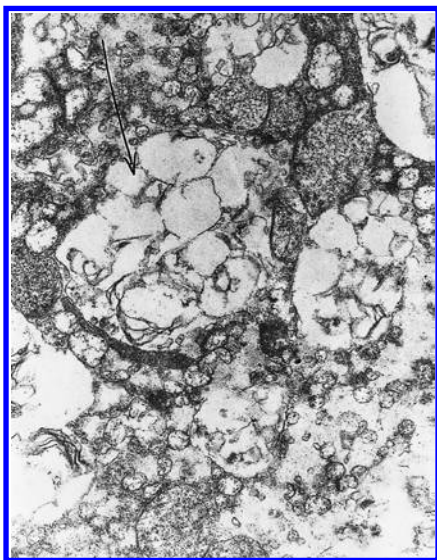


Figure 94.14 In higher magnification ($\times 33,000$) the lucent inclusions contained fragments of membranes and granular material.

composed of the sphingosine C-18 base with a long-chain fatty acid in amide linkage, forming the ceramide moiety, linked to choline monophosphate (Figure 94.1). Levels in affected patients are enormously increased, especially in tissues rich in reticuloendothelial cells [69, 70]. Type A

patients accumulate sphingomyelin in the brain [21, 71], while type B patients generally do not [21]. There is also storage of cholesterol in the liver of patients [69], and this tends to be more in tissues of type A than of type B patients. The other compound that accumulates in the viscera is bis(monacylglycero)phosphate [72].

The molecular genetics of Niemann–Pick disease proceeded rapidly once the gene was sequenced and DNA probes became available. Three common mutations were found in Ashkenazi type A patients [16, 73, 74] that account for 92 percent of the mutant alleles studied [17]. Two are point mutations in exons 6 and 2, R496L (an arginine to leucine change) and L302P (a leucine to proline). The third is a single-base deletion in exon 2 that creates a frameshift (fsP 330) that leads to a stop-codon. The R496L mutation occurred at a CpG dinucleotide, where mutation is common. The two point mutations have been found in patients homozygous for each and in compounds. The R496L mutation has been found in only one of 20 non-Jewish, type A patients; the other two mutations in none. Interestingly, each of the common type A mutations, R476L, L302P, and fsP330 has been found along with a type B mutation in Ashkenazi patients with a type B phenotype [12, 18], underscoring the artificiality of these old classifications. A small number of mutations have been identified in non-Jewish type A patients, each unique to the family in which it was found [75–78]. Four were single-base substitutions; one a nonsense mutation; one was a single-base deletion which caused a frameshift; and one was a splice site change. Two of the point mutations were in adjacent codons in exon 3.

Among type B patients a three-base deletion removing an arginine at 608 ($\Delta R608$) [12, 18] was found in about 12 percent of a large population of type B patients. In homozygous form, patients had mild disease. It also predicts mild disease in compounds with other genes, including the five Ashkenazi Jewish type B patients who carried type A genes in the other allele. This mutation was found in approximately 90 percent of North African Arabs with splenomegaly [79]. In the study of growth restriction in type B children [54], the children homozygous for $\Delta R608$ were of normal height and weight. The S436R (a serine to arginine) also was associated with mild disease in a 19-year-old Japanese patient [76]. L137P, A196P, and R474W were also associated with mild disease [11].

Expression studies [73, 75] indicated that the $\Delta R608$ and other mutations found in milder type B phenotype expressed considerable catalytic activity, while mutations that caused premature stop-codons expressed no catalytic activity in COS cells.

Among the Saudi Arabian patients, some 85 percent of alleles carried the two mutations H421Y and K576N [11]; 11 patients were homozygous for the former and two for the latter. These mutations led to early onset and early demise. All had pulmonary disease.

Niemann–Pick type B is relatively common in Turkish patients, in whom three mutations (L137P, fsP189, and

L549P) accounted for about 75 percent of the alleles [11]. L137P was consistent with quite mild disease in homozygotes and heterozygotes. The A196P mutation, found to be common in patients of Scottish heritage, appeared to convey mild disease even when in compound with a null mutation.

The phenotype–genotype correlations are useful in counseling the parents of newly diagnosed patients, at least in the populations where mutations are common. They are also useful for prenatal diagnosis and carrier detection in any family in which the precise mutation is known, or in an ethnic group in which a small number of mutations is common.

TREATMENT

A variety of transplantations have been made, including liver and amniotic cells, without evident change. Bone marrow transplantation has been reported as being without effect on the neurologic picture of the type A disease [80]. However, it should be of considerable advantage in type B patients because in type B [81] and in type A patients it has been observed that liver and spleen size decreased following transplantation, and improvement in lung infiltration was documented roentgenographically. Both enzyme replacement and gene therapy are under active exploration and animal models of sphingomyelinase deficiency are available.

REFERENCES

1. Niemann A. Ein unbekanntes Krankheitsbild. *Jahrb Kinderheilkd* 1914; **79**: 1.
2. Pick L. Über die lipoidzellige Splenohepatomegalie typus Niemann-Pick als Stoffwechselekrankung. *Med Klin* 1927; **23**: 1483.
3. Pick L. II. Niemann-Pick's disease and other forms of so-called xanthomatosis. *Am J Med Sci* 1933; **185**: 601.
4. Pflander U. La maladie de Niemann-Pick dans le cadre des lipidoses. *Schweiz Med Wochenschr* 1946; **76**: 1128.
5. Crocker AC, Farber S. Niemann-Pick disease: a review of eighteen patients. *Medicine (Balt)* 1958; **37**: 1.
6. Crocker AC. The cerebral defect in Tay-Sachs disease and Niemann-Pick disease. *J Neurochem* 1961; **7**: 69.
7. Klenk E. Über die Natur der Phosphatide der Milz bei Niemann-Pickchen Krankheit. *Z Physiol Chem* 1934; **229**: 151.
8. Brady RO, Kanfer JN, Mock MB, Frederickson DS. The metabolism of sphingomyelin. II. Evidence of an enzymatic deficiency in Niemann-Pick disease. *Proc Natl Acad Sci USA* 1966; **55**: 366.
9. Schneider PB, Kennedy EP. Sphingomyelinase in normal human spleens and in spleens from subjects with Niemann-Pick disease. *J Lipid Res* 1967; **8**: 202.
10. Schettler G, Kahlke W. *Niemann-Pick Disease*. New York: Springer-Verlag, 1967.
11. Simonaro CM, Desnick RJ, McGovern MM *et al*. The demographics and distribution of type B Niemann-Pick disease: novel mutations lead to new genotype/phenotype correlations. *Am J Hum Genet* 2002; **71**: 1413.
12. Besley GTN, Hoogbeem AJM, Hoogveen A *et al*. Somatic cell hybridization studies showing different gene mutations in Niemann-Pick variants. *Hum Genet* 1980; **54**: 409.
13. Quintern L, Schuchman EH, Levran O *et al*. Isolation of cDNA clones encoding human acid sphingomyelinase. Occurrence of alternatively spliced transcripts. *EMBO J* 1989; **8**: 2469.
14. Schuchman EH, Levran O, Desnick RJ. Structural organization and complete nucleotide sequence of the gene encoding human acid sphingomyelinase. *Genomics* 1992; **12**: 197.
15. Pereira L, Desnick RJ, Adler D *et al*. Regional assessment of the human acid sphingomyelinase gene by PCR analysis of somatic cell hybrids and *in situ* hybridization to 11p151-p154. *Genomics* 1991; **9**: 229.
16. Levran O, Desnick RJ, Schuchman EH. Niemann-Pick disease: a common mutation in Ashkenazi Jewish individuals results in the type A and B forms. *Proc Natl Acad Sci USA* 1991; **88**: 3748.
17. Levran O, Desnick RJ, Schuchman EH. Niemann-Pick type B disease: identification of a single codon deletion in the acid sphingomyelinase gene and genotype/phenotype correlations in type A and B patients. *J Clin Invest* 1991; **88**: 806.
18. Frederickson DS, Sloan HR. Sphingomyelin lipidosis: Niemann-Pick disease. In: Stanbury JB, Wyngaarden JB, Frederickson DS (eds). *The Metabolic Basis of Inherited Disease*, 3rd edn. New York: McGraw Hill, 1972: 783.
19. Elleder M, Jirasek A. International symposium on Niemann-Pick disease. *Eur J Pediatr* 1983; **140**: 90.
20. Spence M, Callahan J. *Sphingomyelin-Cholesterol Lipidoses: The Niemann-Pick Group of Diseases*, vol. 2, 6th edn. New York: McGraw-Hill, 1989.
21. Meizner I, Levy A, Carmi R, Robinson C. Niemann-Pick disease associated with nonimmune hydrops fetalis. *Am J Obstet Gynecol* 1990; **163**: 128.
22. Schneider EL, Ellis WG, Brady RO *et al*. Prenatal Niemann-Pick disease: biochemical and histologic examination of a 19-gestational-week fetus. *Pediatr Res* 1972; **6**: 720.
23. Sarrut S, Belamich P. Etude du placenta dans trois observations de dyslipidose à révélation neonatale. *Arch Anat Cytol Pathol* 1983; **31**: 187.
24. Schoenfeld A, Abramovici A, Klibanski C, Ovadia J. Placental ultrasonographic biochemical and histochemical studies in human fetuses affected with Niemann-Pick disease type A. *Placenta* 1985; **6**: 33.
25. Tamaru J, Iwasaki I, Horie H *et al*. Niemann-Pick disease associated with liver disorders. *Acta Pathol Jap* 1985; **35**: 1267.
26. Smith WE, Kahler SG, Frush DP *et al*. Hepatic storage of glycogen in Niemann-Pick disease type B. *J Pediatr* 2001; **138**: 946.
27. Balint JH, Nyhan WL, Lietman P, Turner PH. Lipid patterns in Niemann-Pick disease. *J Lab Clin Med* 1961; **58**: 548.
28. Grunbaum M. The roentgenographic findings in the acute neuronopathic form of Niemann-Pick disease. *Br J Radiol* 1976; **49**: 1018.

29. Dinari G, Rosenbach Y, Grunebaum M *et al.* Gastrointestinal manifestations of Niemann–Pick disease. *Enzyme* 1980; **25**: 407.
30. Yamano T, Shimada M, Okada S *et al.* Ultrastructural study of biopsy specimens of rectal mucosa: its use in neuronal storage diseases. *Arch Pathol Lab Med* 1982; **106**: 673.
31. Cogan DG, Kuwabara T. The sphingolipidoses and the eye. *Arch Ophthalmol* 1968; **79**: 437.
32. Cogan DG, Chu FC, Barranger JA, Gregg RE. Macular halo syndrome. Variant of Niemann–Pick disease. *Arch Ophthalmol* 1983; **101**: 1698.
33. Lipson MH, O'Donnell J, Callahan JW *et al.* Ocular involvement in Niemann–Pick disease Type B. *J Pediatr* 1986; **108**: 582.
34. Matthews JD, Weiter JJ, Kolodney EH. Macular halos associated with Niemann–Pick type B disease. *Ophthalmology* 1986; **93**: 933.
35. Markini MK, Gergen P, Akhtar M, Ghandour M. Niemann–Pick disease: report of a case with skin involvement. *Am J Dis Child* 1982; **136**: 650.
36. Maurer LE. Niemann–Pick's disease a report of four cases. *Rocky Mtn Med J* 1941; **38**: 460.
37. Blankenship RM, Greenburg BR, Lucas RN *et al.* Familial sea-blue histiocytes with acid phosphatemia. A syndrome resembling Gaucher disease: the Lewis variant. *J Am Med Assoc* 1973; **225**: 54.
38. Chan WC, Lai KS, Todd D. Adult Niemann–Pick disease – a case report. *J Pathol* 1977; **121**: 177.
39. Dawson PG, Dawson G. Adult Niemann–Pick disease with sea-blue histiocytes in the spleen. *Hum Pathol* 1982; **13**: 1115.
40. Vanier MT, Rousson R, Garcia I *et al.* Biochemical studies in Niemann–Pick disease. III *In vitro* and *in vivo* assays of sphingomyelin degradation in cultured skin fibroblasts and amniotic fluid cells for the diagnosis of the various forms of the disease. *Clin Genet* 1985; **27**: 20.
41. Landas S, Foucar K, Sando GN *et al.* Adult Niemann–Pick disease masquerading as sea-blue histiocyte syndrome: report of a case confirmed by lipid analysis and enzyme assays. *Am J Hematol* 1985; **20**: 391.
42. Elleder M, Cihula J. Niemann–Pick disease (variation in sphingomyelinase deficient group): neurovisceral phenotype (A) with an abnormally protracted clinical course and variable expression of neurological symptomatology in three siblings. *Eur J Pediatr* 1981; **140**: 323.
43. Sogawa H, Horino K, Nakamura F *et al.* Chronic Niemann–Pick disease with sphingomyelinase deficiency in two brothers with mental retardation. *Eur J Pediatr* 1978; **128**: 235.
44. Hammersen G, Oppermann HC, Harms E *et al.* Oculo-neural involvement in an enzymatically proven case of Niemann–Pick disease type B. *Eur J Pediatr* 1979; **137**: 77.
45. Shah MD, Desai AP, Jain MK *et al.* Niemann–Pick disease type B with oculoneural involvement. *Indian Pediatr* 1983; **20**: 521.
46. Harzer K, Ruprecht KW, Seuffer-Schulze D, Jans U. Morbus Niemann–Pick type B: Enzymatisch Gesichert mit unerwarteter retinaler Beteiligung. *Albrecht Von Graefes Arch Klin Ophthalmol* 1978; **206**: 79.
47. Lowe D, Martin F, Sarks J. Ocular manifestations of adult Niemann–Pick disease: a case report. *Aust NZ J Ophthalmol* 1986; **14**: 41.
48. Takada G, Satoh W, Komatsu K *et al.* Transitory type of sphingomyelinase deficient Niemann–Pick disease: clinical and morphological studies and follow-up of two sisters. *Tohoku J Exp Med* 1987; **153**: 27.
49. Al-Essa MA, Ozand PT. Lysosomal storage disease. Alessa MA, Ozand PT (eds). *Atlas of Common Lysosomal and Peroxisomal Disorders*. Riyadh: King Faisal Specialist Hospital and Research Centre, 1999: 1.
50. Roy D, Oqiel SA. What's your diagnosis? *Ann Saudi Med* 2001; **21**: 127.
51. Conolly CE, Kennedy SM. Primary biliary atresia and Niemann–Pick disease. *Hum Pathol* 1984; **15**: 97 (letter).
52. Tassoni JP, Fawaz KA, Johnson DE. Cirrhosis and portal hypertension in a patient with adult Niemann–Pick disease. *Gastroenterology* 1991; **100**: 567.
53. Lever AML, Ryder JB. Cor pulmonale in adult secondary to Niemann–Pick disease. *Thorax* 1983; **38**: 873.
54. Wasserstein MP, Larkin AE, Glass RB *et al.* Growth restriction in children with type B Niemann–Pick disease. *J Pediatr* 2003; **142**: 424.
55. Bloom W. Splenomegaly (type Gaucher) and lipid-histiocytosis (type Niemann). *Am J Pathol* 1925; **1**: 595.
56. Bloom W. The histogenesis of essential lipid histiocytosis (Niemann–Pick disease). *Arch Pathol* 1928; **6**: 827.
57. Kanfer JN, Young OM, Shapiro D, Brady RO. The metabolism of sphingomyelin. I Purification and properties of a sphingomyelin-cleaving enzyme from rat liver tissue. *J Biol Chem* 1966; **241**: 1081.
58. Quintern LE, Weitz G, Nehr Korn H *et al.* Acid sphingomyelinase from human urine. Purification and characterization. *Biochim Biophys Acta* 1987; **922**: 323.
59. Quintern LE, Zenk TS, Sandhoff K. The urine from patients with peritonitis as a rich source for purifying human acid sphingomyelinase and other lysosomal enzymes. *Biochim Biophys Acta* 1989; **1003**: 121.
60. Callahan JW, Khalil M. Sphingomyelinases in human tissues. III Expression of Niemann–Pick disease and other lysosomal storage diseases. *Biochim Biophys Acta* 1975; **754**: 82.
61. Gatt S, Dinur T, Kopolvic J. Niemann–Pick disease: presence of the magnesium-dependent sphingomyelinase in brain of infantile form of the disease. *J Neurochem* 1978; **30**: 917.
62. Brady RO. The abnormal biochemistry of inherited disorders of lipid metabolism. *Fed Proc* 1973; **32**: 1660.
63. Kudoh T, Velkoff MA, Wenger DA. Uptake and metabolism of radioactively labeled sphingomyelin in cultured skin fibroblasts from controls and patients with Niemann–Pick disease and other lysosomal storage diseases. *Biochim Biophys Acta* 1983; **754**: 82.
64. Kampine JP, Brady RO, Kanfer JN. Diagnosis of Gaucher's disease and Niemann–Pick disease with small samples of venous blood. *Science* 1967; **155**: 86.
65. Zitman D, Chazan S, Klibansky C. Sphingomyelinase activity levels in human peripheral blood leukocytes using [3H] sphingomyelin as substrate: study of heterozygotes and homozygotes for Niemann–Pick disease variants. *Clin Chim Acta* 1978; **86**: 37.

66. Gal AE, Brady RO, Hibbert SR, Pentchev PG. A practical chromogenic procedure for the detection of homozygotes and heterozygous carriers of Niemann-Pick disease. *N Engl J Med* 1975; **293**: 632.
67. Vanier MT, Boue J, Dumaz Y. Niemann-Pick disease type B: first-trimester prenatal diagnosis on chorionic villi and biochemical study of a foetus at 12 weeks of development. *Clin Genet* 1985; **28**: 348.
68. Maziere JC, Maziere C, Hosli P. An ultramicrochemical assay for sphingomyelinase: rapid prenatal diagnosis of a fetus at risk for Niemann-Pick disease. *Monogr Hum Genet* 1978; **9**: 198.
69. Vanier MT. Biochemical studies in Niemann-Pick disease. I. Major sphingolipids in liver and spleen. *Biochim Biophys Acta* 1983; **750**: 178.
70. Rao BG, Spence MW. Niemann-Pick disease: lipid analyses and studies on sphingomyelinases. *Ann Neurol* 1977; **1**: 385.
71. Besley GTN, Elleder M. Enzyme activities and phospholipid storage patterns in brain and spleen samples from Niemann-Pick disease variants: a comparison of neuropathic and non-neuropathic forms. *J Inherit Metab Dis* 1986; **9**: 59.
72. Rouser G, Kritchevsky G, Yamamoto A *et al.* Accumulation of a glycerolphospholipid in classical Niemann-Pick disease. *Lipids* 1968; **3**: 287.
73. Levran O, Desnick RJ, Schuchman EH. A common missense mutation (L302P) in Ashkenazi Jewish Type A Niemann-Pick disease patients. Transient expression studies demonstrate the causative nature of the two common Ashkenazi Jewish Niemann-Pick disease mutations. *Blood* 1992; **80**: 2081.
74. Levran O, Desnick RJ, Schuchman EH. Type A Niemann-Pick disease: a frame-shift mutation in the acid sphingomyelinase gene (fsP330) occurs in about 8 percent of Ashkenazi Jewish alleles. *Hum Mutat* 1993; **2**: 213.
75. Takahashi T, Suchi M, Desnick RJ *et al.* Identification and expression of five new mutations in the acid sphingomyelinase gene which cause types A and B Niemann-Pick disease. Molecular evidence for genetic heterogeneity in the neuronopathic and non-neuronopathic forms. *J Biol Chem* 1992; **267**: 1255.
76. Takahashi T, Desnick RJ, Takada G, Schuchman EH. Identification of a missense mutation (S436R) in the acid sphingomyelinase gene from a Japanese patient with type B Niemann-Pick disease. *Hum Mutat* 1992; **1**: 70.
77. Ferlinz K, Hurwitz R, Sandhoff K. Molecular basis of acid sphingomyelinase deficiency in a patient with Niemann-Pick disease Type A. *Biochem Biophys Res Commun* 1991; **179**: 1187.
78. Levran O, Desnick RJ, Schuchman EH. Identification of a 3' acceptor splice site mutation (g2610c) in the acid sphingomyelinase gene of patients with types A and B Niemann-Pick disease. *Hum Mol Genet* 1993; **2**: 205.
79. Vanier MT, Ferlinz K, Rousson R *et al.* Deletion of arginine (608) in acid sphingomyelinase is the prevalent mutation among Niemann-Pick disease type B patients from North Africa. *Hum Genet* 1993; **92**: 325.
80. Bayeuer E, August CS, Kaman N *et al.* Bone marrow transplantation for Niemann-Pick disease (Type 1A). *Bone Marrow Transplant* 1992; **10**: 83.
81. Vellodi A, Hobbs JR, O'Donnel NM *et al.* Treatment of Niemann-Pick disease type B by allogenic bone marrow transplantation. *Br Med J* 1987; **295**: 1375.

Niemann-Pick type C disease/cholesterol-processing abnormality

Introduction	718	Treatment	722
Clinical abnormalities	718	References	722
Genetics and pathogenesis	721		

MAJOR PHENOTYPIC EXPRESSION

Paralysis of vertical gaze, ataxia, dystonia, hypotonia, prolonged neonatal icterus, hepatosplenomegaly, dementia, foam cells, lysosomal storage of cholesterol, and impaired cellular esterification of cholesterol.

INTRODUCTION

Niemann-Pick type C was first described in the review by Crocker and Farber [1] of their 18 patients with Niemann-Pick disease; the classic features of paralysis of upward gaze, ataxia, dystonia, spasticity, and seizures were clearly described in one of the patients, all of whom had the characteristic foam cells and storage of sphingomyelin. They classified Niemann-Pick into types A and B (Chapter 94), C and D, which is now known to be a variant of C, described in a French-Acadian isolate in Nova Scotia [2]. When the defect in sphingomyelinase was found in types A and B by Brady and his colleagues [3], it became evident that this enzyme was normal in type C [4]. Pentchev and colleagues [5, 6] discovered defective esterification of exogenous cholesterol in the mutant BALB/c mouse. This group then showed that the same faulty regulation of cholesterol processing and storage was present in cultured fibroblasts from patients with Niemann-Pick type C disease [7]. The study of complementation in somatic cell hybrids indicated that type D was allelic with type C on one gene, and clearly separate from types A and B [8].

Complementation studies of type C indicated the presence of two complementation groups [9, 10]. In 95 percent of patients studied, the gene (*NPC1*) was mapped to chromosome 18q11–12 [11, 12]. The smaller group of patients considered to have a defective *NPC2* gene [10] have been reported [13] to have abnormalities in the gene *HE1* on chromosome 14q24.3, which codes for a lysosomal

cholesterol-binding protein [14]. A number of mutations in the *NPC1* gene have been found [12].

In Niemann-Pick type C disease, the trafficking of lipid within the cell leads to accumulation of unesterified cholesterol in lysosomes and late endosomes. The NPC1 protein, which is defective in this disease, is a multifunctional protein, normally situated in a unique late endosomal compartment that becomes enriched with low density lipoprotein (LDL) cholesterol [15, 16]. It is thought that this protein and that of the C2 gene facilitate the egress of cholesterol from late endosomes or lysosomes to the Golgi, endoplasmic reticulum and plasma membrane [17–19]. Ultimately, one would hope that this disease would be known by a name that more closely reflects the fundamental defect.

CLINICAL ABNORMALITIES

The classic patient with this disease appears normal at birth, and while there is a fatal form of the disease, and some patients may have neonatal manifestations of hepatosplenomegaly, jaundice, or hepatic dysfunction, isolated splenomegaly may be the only manifestation for as long as seven years before neurologic signs become apparent [20]. The usual onset is with neurological manifestations between the ages of three and 13 years (Figures 95.1, 95.2, and 95.3) [21].

There may be a tremor, clumsiness, or progressive



Figure 95.1 A three-year-old with Niemann-Pick disease type C. Illustrated is the hepatomegaly and even more massive splenomegaly. Early development was slow, but she walked by two years; by three years she could no longer walk. She had hypertonia. She died at five years. (Illustration kindly provided by Dr Philip Benson.)



Figure 95.3 GC: Development was at seven years. Storage material was evident in conjunctival cells, and typical Niemann-Pick cells were found in the marrow. Cultured fibroblasts were examined by Dr Roscoe Brady who found defective transport of cholesterol out of the Golgi and lysosomes.



Figure 95.2 GC: A nine-year-old Costa Rican girl with Niemann-Pick disease type C. The first symptom was a tremor at seven years. She then developed ataxia. She had paralysis of upward gaze and weakness of peripheral muscles. Liver and spleen were not enlarged.



Figure 95.4 GS: A 29-year-old woman with Niemann-Pick type C disease. She was wheelchair-bound, athetoid, dystonic, and dysphagic. She had expressive aphasia. A sibling had died at 32 years of age, and ballooned cells were found in the brain and other organs.

ataxia. School performance may suffer, as ability to concentrate is lost. On examination, the key finding is vertical supranuclear ophthalmoplegia (Figures 95.4, 95.5, and 95.6) [22]. Impairment of upward gaze may be the first clinical finding. Downward gaze may also be impaired. Speech is dysarthric. Dystonia develops with posturing on movement and becomes progressively more pronounced and generalized [23]. Seizures may develop



Figure 95.5 GS: Paralysis of upward gaze. She was attempting to look up at the examiner's fingers.



Figure 95.6 GS: Ophthalmoplegia also involved inability of downward gaze. The hand illustrates the athetoid movement induced by the effort. She could still move her gaze to each side.

and may be difficult to control. Neurologic dysfunction is progressive. Ophthalmoplegia may ultimately be complete. Hepatosplenomegaly may be detected in childhood (Figures 95.1, 95.2, and 95.3), but with growth this organomegaly may recede. An unusual, not common feature, referred to as gelastic cataplexy, may manifest in nods of the head, or there may be a complete collapse in response to a humerous stimulus [24, 25]. The sudden loss of postural tone may lead to falls and injury. Abnormal behavior may progress to dementia, amentia, or psychosis. The patient becomes wheelchair-bound, and then bedridden. Some have become blind. Early hypotonia may be displaced by spasticity or rigidity. Dysphagia develops, along with drooling and aspiration pneumonia. Death may ensue for these reasons or inanition.

The disease is highly variable in presentation and accordingly difficult to recognize and often diagnosed late. Among the earliest onset, most severe disease, some patients have presented with progressive hepatic dysfunction leading to death well before the onset of neurologic disease

[26–29]. Patients have also been reported with fetal ascites [30, 31]; most died in infancy; this disease has been listed as the second most common cause of liver disease in the UK, after α -1-antitrypsin deficiency (Chapter 105) [32]. Of four patients, two died of hepatic failure and another from respiratory failure with foam cells in the interstitial tissue of the lungs [33]. One child presented at four months with respiratory manifestations [20].

Some patients have had delayed development from infancy [25, 34–36], but neurodegeneration began at about three years. Others have had hypotonia.

A variety of hepatic presentations has been observed. The most common is neonatal cholestatic jaundice [21] and this usually disappears spontaneously early, but there may be prolonged, severe jaundice with elevated conjugated bilirubin [37]. Neonatal jaundice has been reported with early-onset rapidly progressive neurologic disease [38]. Others have developed hepatic failure in the absence of neurologic disease.

A number of late-onset or adult presentations have also been reported [22, 39–44]. Some of the patients have had exclusively psychiatric symptoms, psychosis or progressive cognitive loss and dementia. Most adults have developed paralysis of vertical gaze, but some have not, and some have complained that their eyes became stuck on looking up. Others have developed ataxia and pyramidal and extrapyramidal signs, and the neurologic picture of the classic disease [44]. Cerebral atrophy may be evident on neuroimaging. Some have had neonatal hepatitis and then seemed to be well until the development of psychotic symptoms in adolescence or adulthood [43]. A past history of splenomegaly may be another clue to the nature of the disease. Some adults have had a nonneuropathic presentation [43, 44], including a man whose large spleen was ruptured in a traffic accident at 46 years and found to contain foam cells [44]; four years later he was asymptomatic and had a normal neurologic examination.

A fetal form of the disease was reported [45] in five families, three of them consanguineous. Two were diagnosed prenatally by study of amniocytes after developing splenomegaly and ascites. Two had an affected sib. Prognosis was bad. One died *in utero*; one was terminated during pregnancy; four died within seven months of cholestatic disease, and the seventh had rapid neurologic deterioration at ten months.

The phenotype of patients with NPC2 disease appears to be indistinguishable clinically or biochemically from the more common type 1 disease, but severe pulmonary involvement has been observed [46].

The diagnosis of a neuropilidosis is usually made on the basis of the characteristic foam cells. The usual source is a bone marrow aspirate [22, 47, 48]. Sea-blue histiocytes may be seen [47], as well as the foam cells (Chapter 94, Figure 94.10). The cells stain with periodic acid-Schiff stain, and strongly so with the Schultz stain for cholesterol and the acid phosphatase stain [48–53]. Foam cells may be found on conjunctival biopsy and skin biopsy [54]. In

the electron microscope, there are numerous concentric electron-dense inclusions and electron-lucent vacuoles [39, 49, 50, 55].

Pathologic examination indicates the presence of foamy storage cells, particularly in the liver, spleen, tonsils, and lymph nodes [51, 52]. Early infantile-onset disease is associated with an appearance of giant cell hepatitis and cholestasis [26, 29, 38, 39].

Storage in neurons is seen widely in the cerebrum, the cerebellum, and the retina [50, 56–58], and the brain is atrophic. Lamellar inclusions may resemble the membranous cytoplasmic bodies of the gangliosidosis or zebra bodies. Crystalline structures were observed [59] in a 20-week-old fetus, suggesting crystalline cholesterol. Similar structures were found in a murine model of the disease [60].

GENETICS AND PATHOGENESIS

Niemann-Pick type C is an autosomal recessive lysosomal storage disease [61]. Despite considerable clinical variability, one complementation group represents a majority of the patients [9, 10, 61]. The gene (*NPC1*) was mapped to chromosome 18 (q11–12) [11]. In a study of 32 unrelated patients [10], 27 fell into this NPC1 group. Five patients fell into the second NPC2 group. Patients in the first group illustrated the entire spectrum of disease. In general, phenotypes within any family were quite similar, except that fatal neonatal disease was found in families in which others had classic neurologic disease [61].

The disease has been seen widely throughout the world [62]. A genetic isolate of French-Acadians in Nova Scotia was originally called type D [2]. In their county the incidence was one percent, and carrier frequency was 10–26 percent [63]. Another isolate was found in Hispanic-Americans in south Colorado [47]. In France and England the disease was found to be as frequent as Niemann-Pick types A and B combined [64].

The *NPC1* gene contains 25 exons over 47 kb [65]. It predicts a protein of 1278 amino acids [12]. The protein appears to be a permease which acts as a transmembrane efflux pump [14]. It has extensive homology to other proteins, including the murine ortholog, and to patched, the defect in the basal cell nevus syndrome [66], which is also related to the sonic hedgehog signaling pathway; and to proteins involved in cholesterol homeostasis. Eight mutations were originally found in five patients, two deletions, one insertion, and five missense mutations. In Japan, mutations identified included two splicing abnormalities [67]. In the Nova Scotian French isolate the defect was a missense mutation c.G3097T which led to p.G992W [68]. A considerable number and variety of unique mutations have now been found [69]. The only common Caucasian mutation is p.I1061T, when homozygous leads to a juvenile neurological phenotype [70]. This mutation has been found in the Hispanic-

American isolate in Colorado and New Mexico. In most populations compound heterozygosity is the rule [71].

The gene for NPC2 was mapped to chromosome 14q24.3 [72]. In studies of the epididymal secretory protein HE1, it was reasoned from the facts that it bound cholesterol and was lysosomal and that it might be relevant to Niemann-Pick C disease. Activity of HE1 was found to be undetectable in fibroblasts from patients with NPC2. Mutations were found in the HE1 gene. Homozygous mutations were found in six patients with NPC2 [73]. Among the mutations only p.P1205 led to detectable quantities of immunoprotein. IVS1 +2t>c led to a number of transcripts, none of them normally spliced. Genotype phenotype correlations appeared to be good in 22 families with mutations.

Animal models of Niemann-Pick type C disease have been found: a feline and two murine models [5, 6, 74]. The mice were ataxic and had typical foam cells like those of human patients. There was marked cerebellar loss of Purkinje cells.

The fundamental defect in the mice and in humans was in the transportation system for cholesterol in cells [5–7]. Cultured fibroblasts of patients and affected mice were deficient in their ability to make cholesteryl esters from endocytically taken-up exogenous LDL cholesterol [6]. This leads to lysosomal accumulation of cholesterol [7, 75–77]. The enzymes involved in cholesterol esterification, such as acyl-CoA:cholesterol acyl transferase (ACAT), are normal in these cells, and treatment of the cells with 25-hydroxycholesterol reverses the abnormality of cellular regulation of exogenous cholesterol [78].

The abnormality is conveniently demonstrated with filipin, a fluorescent probe that detects unesterified cholesterol [79]. Following LDL uptake by type C cells, the lysosomal vacuoles light up; this is the procedure that has facilitated complementation studies [10]. Endogenously synthesized cholesterol is processed normally because it does not end up in lysosomes [17]. These observations served to focus attention on systems for transport out of lysosomes.

The diagnosis of Niemann-Pick type C disease is currently made in cultured fibroblasts by demonstration of both impaired cholesterol esterification and the positive filipin test for accumulation of free-cholesterol [80–82]. A considerable amount of heterogeneity has been observed in these tests, ranging from mild to severe changes [83]. A majority of patients (86 percent) have cholesterol esterification rates less than 10 percent of normal [81]. Some are very mildly affected and some intermediate, but correlations of this biochemistry with phenotype have not been clear. Assay by filipin staining is more broadly effective, particularly in the diagnosis of variant patients [21, 81]. It is also more specific, because abnormal esterification occurs in other disorders, such as I cell disease (Chapter 83), familial cholesterolemia (Chapter 85), and acid lipase deficiency.

An aid to diagnosis may be obtained by assay of the

activity of chitotriosidase [43]. This activity is significantly increased in Gaucher disease [84]. It may be moderately elevated in Niemann-Pick type C disease and some other lysosomal storage diseases, but it may be normal too [43].

Heterozygote detection is unreliable, although some have foamy cells in marrow or skin biopsies [85, 86] and intermediate levels of cholesterol esterification are found in about half of obligate heterozygotes [21, 81, 82, 87]. Prenatal diagnosis has been carried out by biochemical testing in cultured amniocytes and chorionic villus cells [88, 89]. Thirteen affected fetuses were found in 37 pregnancies at risk [89]. Only the families with the most severe chemical expression appear to be reliable candidates for biochemical prenatal diagnosis. The extensive molecular heterogeneity makes mutational analysis formidable, except in population isolates or in families in which the mutation is known. In these instances this is the method of choice for heterozygote detection and prenatal diagnosis [69, 71].

The adenosine triphosphatase (ATP)-binding cassette transporter A1 (ABCA1) is also upregulated in response to increased cellular cholesterol, leading to high density lipoprotein (HDL) particle formation. Mutations in this ABCA1 lead to increased intracellular cholesterol and very low levels of HDL in Tangier disease [90]. Mutations in NPC1 appear to impair also the regulation and activity of ABCA1 [91]. Fibroblasts from patients with NPC disease were shown to have decreased efflux of labeled LDL-cholesterol mediated by apolipoprotein A-I. These fibroblasts also displayed diminished ABCA1 mRNA and protein in both basal and cholesterol stimulated states. Furthermore, 17 of 21 patients studied had low levels of HDL-cholesterol. This observation can provide another diagnostic aid in evaluating children for NPC disease.

Despite the large and increasing body of knowledge about the metabolism and transport of cholesterol and other lipids, the pathogenesis of the neurologic features of Niemann-Pick type C disease remains obscure [92]. Experience with cholesterol-lowering drugs and bone marrow transplantation in man, and even combined liver and bone marrow transplantation in the mouse model, none of which were effective in influencing the neurodegeneration [93, 94], indicated clearly that the central nervous system (CNS) is autonomous from the rest of the body. Liver transplantation in a seven-year-old cirrhotic girl restored hepatic function, but failed to reverse neurologic deterioration [95]. A variety of lipids in addition to cholesterol accumulate in the brains of patients with this disease. These include GM2 and GM3 gangliosides, glucosylceramide, and lactosylceramide. There is neuronaxonal dystrophy and neurofibrillary tangles, like those of Alzheimer disease. In Niemann-Pick type C lipid rafts, which occur in the lipid bilayer of the plasma membranes of glia and neurons, accumulate because of defective egress. Approaches to reduce the accumulation of sphingolipid by inhibiting its synthesis have been underway in murine models, and human trials are

planned. N-butyldeoxynojirimycin inhibits the synthesis of GM2 ganglioside [96] and has resulted in reduced ganglioside accumulation in brain, reduced Purkinje cell loss, and modest delay in neurologic disease and death. Similarly, breeding affected mice with mice carrying a mutation in the transferase gene that inhibits synthesis of GM2, GA1, and GA2 indicated that these lipids are not the cause of the neuropathology [97]. Disordered trafficking of lipoprotein cholesterol leads to disordered oxysterol and sterol biosynthesis. In homozygous NPC1 knockout mice, treatment with allopregnanolone and an oxysterol ligand delayed the onset of neurologic disease [98].

TREATMENT

Specific treatment is not available. The promise of gene therapy was raised by successful prevention of neurodegeneration and extension of life span in homozygous npc mice by overexpression of the NPC1 gene targeted to the CNS [99].

Seizures may be controlled with the usual anticonvulsant agents. Protryptilene and clomipramine are useful in cataplexy and sleep problems [100, 101]. Dystonia and tremor may respond to anticholinergic drugs. Supportive care including physical and occupational therapy is important. Support groups are available in the US and in Europe.

A therapeutic trial of butyldeoxynojirimycin (miglustat), found to prolong survival in mice [96], is underway in man. Preliminary data appear to show some benefit [102].

REFERENCES

1. Crocker AC, Farber S. Niemann-Pick disease: a review of eighteen patients. *Medicine (Baltimore)* 1958; **37**: 1.
2. Crocker AC. The cerebral defect in Tay-Sachs disease and Niemann-Pick disease. *J Neurochem* 1961; **7**: 69.
3. Brady RO, Kanfer HJN, Mock MB, Frederickson DS. The metabolism of sphingomyelin. II Evidence of an enzymatic deficiency in Niemann-Pick disease. *Proc Natl Acad Sci USA* 1966; **55**: 366.
4. Schneider PB, Kennedy EP. Sphingomyelinase in normal human spleens and in spleens from subjects with Niemann-Pick disease. *J Lipid Res* 1967; **8**: 202.
5. Pentchev PG, Gal AE, Boothe AD *et al*. A lysosomal storage disorder in mice characterized by dual deficiency of sphingomyelinase and glucocerebrosidase. *Biochem Biophys Acta* 1980; **619**: 669.
6. Pentchev PG, Comly ME, Kruth HS *et al*. The cholesterol storage disorder of the mutant BALB/c mouse. A primary genetic lesion closely linked to defective esterification of exogenously derived cholesterol and its relationship to

- human type C Niemann-Pick disease. *J Biol Chem* 1986; **261**: 2792.
7. Pentchev PG, Comly ME, Kruth HS *et al.* Group C Niemann-Pick disease: faulty regulation of low-density lipoprotein uptake and cholesterol storage in cultured fibroblasts. *FASEB J* 1987; **1**: 40.
8. Besley GT, Hoogeboom AJ, Hoogveen A *et al.* Somatic cell hybridisation studies showing different gene mutations in Niemann-Pick variants. *Hum Genet* 1980; **54**: 409.
9. Steinberg SJ, Ward CP, Fensom AH. Complementation studies in Niemann-Pick disease type C indicate the existence of a second group. *J Med Genet* 1994; **31**: 317.
10. Vanier MT, Millat G. Niemann-Pick disease type C. *Clin Genet* 2003; **64**: 269.
11. Carstea ED, Polymeropoulos MH, Parker CC *et al.* Linkage of Niemann-Pick disease type C to human chromosome 18. *Proc Natl Acad Sci USA* 1993; **90**: 2002.
12. Carstea ED, Morris JA, Coleman KG *et al.* Niemann-Pick C1 disease gene: homology to mediators of cholesterol homeostasis. *Science* 1997; **277**: 228.
13. Naureckiene S, Sleat DE, Lackland H *et al.* Identification of HE1 as the second gene of Niemann-Pick C disease. *Science* 2000; **209**: 2298.
14. Davies JP, Chen FW, Ioannou YA. Transmembrane molecular pump activity of Niemann-Pick C1 protein. *Science* 2000; **290**: 2295.
15. Neufeld EB, Wastney M, Patel S *et al.* The Niemann-Pick C1 protein resides in a vesicular compartment linked to retrograde transport of multiple lysosomal cargo. *J Biol Chem* 1999; **274**: 9627.
16. Garver WS, Heidenreich RA, Erickson RP *et al.* Localization of the murine Niemann-Pick C1 protein to two distinct intracellular compartments. *J Lipid Res* 2000; **41**: 673.
17. Garver WS, Krishnan K, Gallagos JR *et al.* Niemann-Pick C1 protein regulates cholesterol transport to the trans-Golgi network and plasma membrane caveolae. *J Lipid Res* 2002; **43**: 579.
18. Wojtanik KM, Liscum L. The transport of low density lipoprotein-derived cholesterol to the plasma membrane is defective in NPC1 cells. *J Biol Chem* 2003; **278**: 14 850.
19. Vance JE. Lipid imbalance in the neurological disorder, Niemann-Pick C disease. *FEBS Lett* 2006; **580**: 5518.
20. Imrie J, Wraith JE. Isolate splenomegaly as the presenting feature of Niemann-Pick disease type C. *Arch Dis Child* 2000; **84**: 427.
21. Vanier MT, Wenger DA, Comley ME *et al.* Niemann-Pick disease group C: clinical variability and diagnosis based on defective cholesterol esterification. A collaborative study on 70 patients. *Clin Genet* 1988; **33**: 331.
22. Grover WD, Naiman JL. Progressive paresis of vertical gaze in lipid storage disease. *Neurology* 1982; **32**: 1295.
23. Longstreth WT, Daven JR, Farrell DF *et al.* Adult dystonic lipidosis: clinical histologic and biochemical findings of a neurovisceral storage disease. *Neurology* 1982; **32**: 1295.
24. Denoix C, Rodriguez-Lafrasse C, Vanier MT *et al.* Cataplexie révélatrice d'une forme atypique de la maladie de Niemann-Pick type C. *Arch Fr Pediatr* 1991; **48**: 31.
25. Miyake S, Inoue H, Ohtahara S *et al.* A case of Niemann-Pick disease type C with narcolepsy syndrome. *Rinsho Shinkeigaku* 1983; **23**: 44.
26. Rutledge JC. Case 5 Progressive neonatal liver failure due to type C Niemann-Pick disease. *Pediatr Pathol* 1989; **9**: 779.
27. Gonzalez de Dios J, Fernandez Tejada E, Diaz Fernandez MC *et al.* Estado actual de la enfermedad de Niemann-Pick: valoración de seis casos. *An Esp Pediatr* 1990; **32**: 143.
28. Guibaud P, Vanier MT, Malpeuch G *et al.* Forme infantile précoce cholestatique rapidement mortelle de la sphingomyelinase de type C. A propos de deux observations. *Pediatric* 1979; **43**: 103.
29. Jaeken J, Proesmans W, Eggermont E *et al.* Niemann-Pick type C disease and early cholestasis in three brothers. *Acta Paediatr Belg* 1980; **33**: 43.
30. Maconochie JK, Chong S, Mieli-Vergani G *et al.* Fetal ascites: an unusual presentation of Niemann-Pick disease type C. *Arch Dis Child* 1989; **64**: 1391.
31. Manning DJ, Price WI, Pearse RG. Fetal ascites: an unusual presentation of Niemann-Pick disease type C. *Arch Dis Child* 1990; **65**: 335.
32. Mieli-Vergani G, Howard ER, Mowat AP. Liver disease in infancy: A 20-year perspective. *Gut* 1991; **32**(Suppl.): S123.
33. Pin I, Pradines S, Pincemaille O *et al.* Forme respiratoire mortelle de maladie de Niemann-Pick type C. *Arch Fr Pediatr* 1990; **47**: 373.
34. Fensom AH, El Kalla S, Bizzari R *et al.* Clinical presentation and diagnosis of Niemann-Pick disease type C. *Emirates Med J* 1990; **8**: 215.
35. Wiedemann H-R, Debuch H, Lennert K *et al.* Über eine infantil-juvenile subchronisch verlaufende, den Sphingomyelinosen (Niemann-Pick) anzureihende Form der Lipidosen-ein neuer Typ? Klinische pathohistologische elektronmikroskopische und biochemische Untersuchungen. *Z Kinderheilkd* 1972; **112**: 187.
36. Harzer K, Schlote W, Peiffer J *et al.* Neurovisceral lipidosis compatible with Niemann-Pick disease type C: morphological and biochemical studies of a late infantile case and enzyme and lipid assays in a prenatal case of the same family. *Acta Neuropathol (Berl)* 1978; **43**: 97.
37. Coleman RJ, Robb SA, Lake BD *et al.* The diverse neurological features of Niemann-Pick disease Type C: a report of two cases. *Mov Disord* 1988; **3**: 295.
38. Higgins JJ, Paterson MC, Dambrosia JM *et al.* A clinical staging classification for type C Niemann-Pick disease. *Neurology* 1992; **42**: 2286.
39. Ashkenazi A, Yarom G, Gutman A *et al.* Niemann-Pick disease and giant cell transformation of the liver. *Acta Paediatr Scand* 1971; **60**: 285.
40. Wherrett JR, Rewcastle NB. Adult neurovisceral lipidosis. *Clin Res* 1969; **17**: 665.
41. Houroupan DS, Yang SS. Paired helical filaments in neurovisceral lipidosis (juvenile dystonic lipidosis). *Ann Neurol* 1978; **4**: 404.
42. Hulette CM, Earl NL, Anthony DC, Crain BJ. Adult onset Niemann-Pick disease type C presenting with dementia and absent organomegaly. *Clin Neuropathol* 1990; **1**: 293.
43. Imrie J, Vijayaraghaven S, Whitehouse C *et al.* Niemann-Pick disease type C in adults. *J Inherit Metab Dis* 2002; **25**: 491.

44. Fensom AH, Grant AR, Steinberg SJ. Case report: an adult with a non-neuronopathic form of Niemann-Pick C disease. *J Inherit Metab Dis* 1999; **22**: 84.
45. Spiegel R, Raas-Rothschild A, Reish O *et al*. The clinical spectrum of fetal Niemann-Pick type C. *Am J Med Genet A* 2009; **149A**: 446.
46. Schofer O, Mischo B, Puschel W *et al*. Early-lethal pulmonary form of Niemann-Pick type C disease belonging to a second rare genetic complementation group. *Eur J Pediatr* 1998; **157**: 45.
47. Wenger DA, Barth G, Githens JH. Nine cases of sphingomyelin lipidosis a new variant in Spanish-American children. Juvenile variant of Niemann-Pick disease with foamy and sea blue histiocytes. *Am J Dis Child* 1977; **131**: 955.
48. Neville BGR, Lake BD, Stephens R, Sanders MD. A neurovisceral storage disease with vertical supranuclear ophthalmoplegia and its relationship to Niemann-Pick disease. A report of nine patients. *Brain* 1973; **96**: 97.
49. Martin JJ, Lowenthal A, Ceuterick C, Vanier MT. Juvenile dystonic lipidosis (variant of Niemann-Pick type C). *J Neurol Sci* 1984; **66**: 33.
50. Gilbert EF, Callahan J, Visiskul C, Opitz JM. Niemann-Pick disease type C. Pathological, histochemical, ultrastructural and biochemical studies. *Eur J Pediatr* 1981; **136**: 263.
51. Elleder M, Jirasek A, Smid F *et al*. Niemann-Pick disease type C with enhanced glycolipid storage. Report on further case of so-called lactosylceramidosis. *Virchows Arch* 1984; **A402**: 307.
52. Philipart M, Martin L, Martin JJ, Menkes JH. Niemann-Pick disease. Morphologic and biochemical studies in the visceral form with later central nervous system involvement (Crocker's type C). *Arch Neurol* 1969; **20**: 227.
53. Elleder M, Jirasek A, Smid F. Niemann-Pick disease (Crocker's type C). *Acta Neuropathol (Berl)* 1975; **133**: 191.
54. Arsenio-Nunes ML, Goutieres F. Morphological diagnosis of Niemann-Pick disease type C by skin and conjunctival biopsies. *Acta Neuropathol (Berl)* 1981; **7**: 204.
55. Merin S, Livni N, Yatziv S. Conjunctival ultrastructure in Niemann-Pick disease type C. *Am J Ophthalmol* 1980; **90**: 708.
56. Pellissier JF, Hassoun J, Gambarelli D *et al*. Maladie de Niemann-Pick type 'C' de Crocker. Etude ultrastructural d'un cas. *Acta Neuropathol (Berl)* 1976; **34**: 65.
57. Palmer M, Green WR, Maumenee IH *et al*. Niemann-Pick disease – type C. Ocular histopathologic and electronmicroscopic studies. *Arch Ophthalmol* 1985; **103**: 817.
58. Elleder M, Jirasek A, Smid F *et al*. Niemann-Pick disease type C. Study on the nature of cerebral storage process. *Acta Neuropathol (Berl)* 1985; **66**: 325.
59. Dumontel C, Girod C, Dijoud F *et al*. Fetal Niemann-Pick disease type C: ultrastructural and lipid findings in liver and spleen. *Virchows Arch A Pathol Anat Histopathol* 1993; **422**: 253.
60. Higashi Y, Pentchev PG, Murayama S, Suzuki K. Pathology of Niemann-Pick type C: Studies of murine mutants. In: Ikuta F (ed.). *Neuropathology in Brain Research*. Amsterdam: Elsevier Science, 1991: 85.
61. Vanier MT, Pentchev P, Rodriguez-Lafrasse C, Rousson R. Niemann-Pick disease type C: an update. *J Inherit Metab Dis* 1991; **14**: 580.
62. Vanier MT, Wenger DA, Comly ME *et al*. Niemann-Pick disease group C: clinical variability and diagnosis based on defective cholesterol esterification: a collaborative study on 70 patients. *Clin Genet* 1988; **33**: 331.
63. Winsor EJT, Welch JP. Genetic and demographic aspects of Nova Scotia Niemann-Pick disease (type D). *Am J Hum Genet* 1978; **30**: 530.
64. Vanier MT, Rodriguez-Lafrasse C, Rousson R *et al*. Type C Niemann-Pick disease: biochemical aspects and phenotypic heterogeneity. *Dev Neurosci* 1991; **13**: 307.
65. Morris JA, Zhang D, Coleman KG *et al*. The genomic organization and polymorphisms analysis of the human Niemann-Pick C1 gene. *Biochem Biophys Res Commun* 1999; **261**: 493.
66. Johnson RL, Rothman AL, Xie J *et al*. Human homolog of patched a candidate gene for the basal cell nevus syndrome. *Science* 1996; **272**: 1668.
67. Yamamoto T, Nanba E, Ninomiya H *et al*. NPC1 gene mutations in Japanese patients with Niemann-Pick disease type C. *Hum Genet* 1999; **105**: 10.
68. Greer WL, Riddell DC, Gillan TL *et al*. The Nova Scotia (type D) form of Niemann-Pick disease is caused by a G3097® T transversion in NPC1. *Am J Hum Genet* 1998; **63**: 52.
69. Patterson M. Niemann-Pick disease type C. In: *GeneClinics: Clinical Genetic Information Resource* [database online]. University of Washington, Seattle. Last accessed November 2004. Available from: www.geneclinics.org.
70. Millat G, Marçais C, Rafi MA *et al*. Niemann-Pick C1 disease: the I1061T substitution is a frequent mutant allele in patients of Western European descent and correlates with a classic juvenile phenotype. *Am J Hum Genet* 1999; **65**: 1321.
71. Kaminski WE, Klünemann HH, Ibach B *et al*. Identification of novel mutations in the NPC1 gene in German patients with Niemann-Pick C disease. *J Inherit Metab Dis* 2002; **25**: 385.
72. Naureckiene S, Sleat DE, Lackland H *et al*. Identification of HE1 as the second gene of Niemann-Pick C disease. *Science* 2000; **290**: 2298.
73. Verot L, Chikh K, Freydiere E *et al*. Niemann-Pick C disease: functional characterization of three NPC2 mutations and clinical and molecular update on patients with NPC2. *Clin Genet* 2007; **71**: 320.
74. Morris MD, Bhuvaneshwaran C, Shio H, Fowler S. Lysosome storage disorder in NCTR-BALB/c mice. I Description of the disease and genetics. *Am J Pathol* 1982; **108**: 140.
75. Butler JD, Comly ME, Kruth HS. Niemann-Pick variant disorders: comparison of errors of cellular cholesterol homeostasis in group D and group C fibroblasts. *Proc Natl Acad Sci USA* 1987; **84**: 556.
76. Sokol J, Blanchette-Mackie J, Kruth HS *et al*. Type C Niemann-Pick disease. Lysosomal accumulation and defective intracellular mobilization of low density lipoprotein cholesterol. *J Biol Chem* 1988; **263**: 3411.
77. Blanchette-Mackie EJ, Dwyer NK, Amende LM. Type-C Niemann-Pick disease: low density lipoprotein uptake is associated with premature cholesterol accumulation in the Golgi complex and excessive cholesterol storage in lysosomes. *Proc Natl Acad Sci USA* 1988; **85**: 8022.

78. Liscum L, Faust JR. Low density lipoprotein (LDL)-mediated suppression of cholesterol synthesis and LDL uptake is defective in Niemann-Pick fibroblasts. *J Biol Chem* 1987; **262**: 17002.
79. Severs NJ, Robeneck H. Detection of micro-domains in biomembranes. An appraisal of recent developments in freeze-fracture cytochemistry. *Biochem Biophys Acta* 1983; **737**: 373.
80. Vanier MT, Suzuki K. Niemann-Pick diseases. In: Moser HW, Vinken PJ, Bruyn GW (eds). *Neurodystrophies and Neurolipidoses*, Vol 66. Handbook of Clinical Neurology. Amsterdam: Elsevier Science, 1996: 133.
81. Vanier MT, Rodriguez-Lafrasse C, Rousson R *et al*. Type C Niemann-Pick disease: spectrum of phenotypic variation in disruption of intracellular LDL-derived cholesterol processing. *Biochim Biophys Acta* 1991; **1096**: 328.
82. Roff CF, Goldin E, Comly ME *et al*. Niemann-Pick type C disease: deficient intracellular transport of exogenously derived cholesterol. *Am J Hum Genet* 1992; **42**: 593.
83. Argoff CE, Comly ME, Blanchette-Mackie J *et al*. Type C Niemann-Pick disease: cellular uncoupling of cholesterol homeostasis is linked to severity of disruption in the intracellular transport of exogenously derived cholesterol. *Biochem Biophys Acta* 1991; **1096**: 319.
84. Guo Y, He W, Boer AM *et al*. Elevated plasma chitotriosidase activity in various lysosomal storage disorders. *J Inher Metab Dis* 1995; **18**: 717.
85. Frank V, Lasson V. Ophthalmoplegic neuro-lipidosis storage cells in heterozygotes. *Neuropediatrics* 1985; **16**: 3.
86. Ceuterick C, Martin JJ. Niemann-Pick disease type C. Skin biopsies in parents. *Neuropediatrics* 1986; **17**: 111.
87. Kruth HS, Comly ME, Butler JD *et al*. Type C Niemann-Pick disease. Abnormal metabolism of low density lipoprotein in homozygous and heterozygous fibroblasts. *J Biol Chem* 1986; **261**: 16 769.
88. Vanier MT, Rousson RM, Mandon G *et al*. Diagnosis of Niemann-Pick disease type C on chorionic villus cells. *Lancet* 1989; **1**: 104.
89. Vanier MT, Rodriguez-Lafrasse C, Rousson R *et al*. Prenatal diagnosis of Niemann-Pick type C disease: current strategy from an experience of 37 pregnancies at risk. *Am J Hum Genet* 1992; **51**: 111.
90. Bodzioch M, Orsó E, Klucken J *et al*. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. *Nat Genet* 1999; **22**: 347.
91. Choi HY, Karten B, Chan T *et al*. Impaired ABCA1-dependent lipid efflux and hypoalphalipoproteinemia in human Niemann-Pick type C disease. *J Biol Chem* 2003; **278**: 32 569.
92. Vincent I, Bu B, Erickson RP. Understanding Niemann-Pick type C disease: a fat problem. *Curr Opin Neurol* 2003; **16**: 155.
93. Hsu YS, Hwu WL, Huang SF *et al*. Niemann-Pick disease type C (a cellular cholesterol lipidosis) treated by bone marrow transplantation. *Bone Marrow Transplant* 1999; **24**: 103.
94. Yasumizu R, Miyawaki S, Sugiura K *et al*. Allogenic bone marrow-plus-liver transplantation in the C57BL/KsJ SPM/SPM mouse, an animal model of Niemann-Pick disease. *Transplantation* 1990; **49**: 759.
95. Gartner JC, Bergman I, Malatack JJ *et al*. Progression of neurovisceral storage disease and supra-nuclear ophthalmoplegia following orthotopic liver transplantation. *Pediatrics* 1986; **77**: 104.
96. Zervas M, Somers KL, Thrall MA *et al*. Critical role for glycosphingolipids in Niemann-Pick disease type C. *Curr Biol* 2001; **11**: 1283.
97. Liu Y, Wu YP, Wada R *et al*. Alleviation of neuronal ganglioside storage does not improve the clinical course of the Niemann-Pick C disease mouse. *Hum Mol Genet* 2000; **9**: 1087.
98. Langmade SJ, Gale SE, Frolov A *et al*. Pregnane X receptor (PXR) activation: a mechanism for neuroprotection in a mouse model of Niemann-Pick C disease. *Proc Nat Acad Sci* 2006; **103**: 13 807.
99. Loftus SK, Erickson RP, Walkley SU *et al*. Rescue of neurodegeneration in Niemann-Pick C mice by a prion-promoter-driven Npc1 cDNA transgene. *Hum Mol Genet* 2002; **11**: 3107.
100. Kandt RS, Emerson RG, Singer HS *et al*. Cataplexy in variant forms of Niemann-Pick disease. *Ann Neurol* 1982; **12**: 284.
101. Philipart M, Engel J, Zimmerman EG. Gelastic cataplexy in Niemann-Pick disease group C and related variants without generalized sphingomyelinase deficiency. *Ann Neurol* 1983; **14**: 492.
102. Patterson MC, Vecchio D, Prady H *et al*. Miglustat for treatment of Niemann-Pick C disease: a randomised controlled study. *Lancet Neurol* 2007; **6**: 765.

Krabbe disease/galactosylceramide lipidosis/ globoid cell leukodystrophy

Introduction	726	Treatment	730
Clinical abnormalities	726	References	730
Genetics and pathogenesis	729		

MAJOR PHENOTYPIC EXPRESSION

Rapidly progressive central nervous system degenerative disease, characterized by spastic quadriplegia, blindness, deafness, peripheral neuropathy, and pseudobulbar paralysis; diffuse demyelination; massive infiltration with multinucleated globoid cells; deficiency of galactosylceramide β -galactosidase.

INTRODUCTION

The syndrome was first described by Krabbe in 1916 [1]. He reported five patients, of whom four represented two sets of siblings. All were normal at birth, but had rapidly progressive neurologic deterioration from an early onset at 4–6 months until death by the age of 1.5–2 years. In addition to a detailed description of clinical features of the disease, he clearly documented the pathognomonic neuropathologic features of the disorder, including the accumulation of large multinucleated globoid cells in the white matter. Chemical analysis documented the accumulation of cerebroside in these cells [2, 3] and the induction of globoid cells uniquely by the intracerebral administration of galactocerebroside [4, 5]. The enzymatic defect (Figure 96.1) was discovered in 1970 by Suzuki and colleagues [6, 7], in galactosylceramidase (galactosylceramide- β -galactosidase) (EC 3.2.1.46). The cDNA has been cloned [8], and the gene was mapped to

chromosome 14q24.3–32.1 [9, 10]. A considerable number and variety of mutations have been identified [11]. A single mutation, a 30 kb deletion (502Tdel) has accounted for a large number of Northern European, US, and Mexican patients [12, 13].

CLINICAL ABNORMALITIES

Patients with the classic infantile form of the disease appear normal at birth, and they develop normally for the first few months. The first symptoms usually appear between three and six months of age [1, 2]. The earliest manifestations are often irritability and bouts of crying or screaming without apparent cause. The neurodegeneration is then rapidly progressive (Figures 96.2, 96.3, 96.4, 96.5 and 96.6). Universal rigidity of the muscles is the most typical appearance of the patient with this disease. Fists are

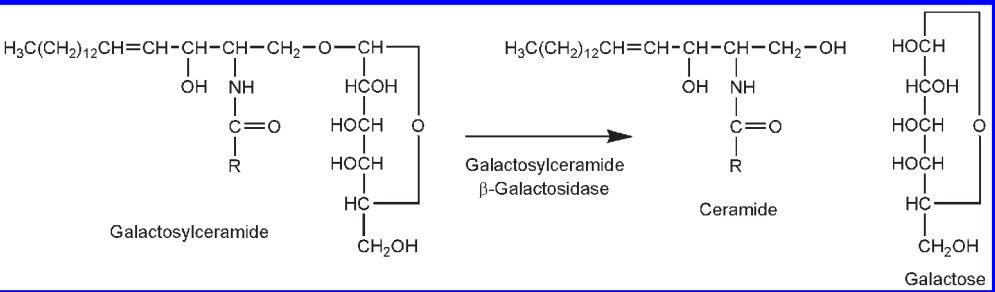


Figure 96.1 The structure of the galactocerebroside, galactosylceramide and the reaction catalyzed by its β -galactosidase. This is the site of defect in the Krabbe disease.



Figure 96.2 SA: A 10-month-old infant with Krabbe disease. This was a modified tonic neck reflex. She was very irritable and had hypertonia. Deep tendon reflexes were exaggerated. She had begun to have trouble handling her secretions.



Figure 96.4 SA: At nine months. The expression was blank and the fists clenched.

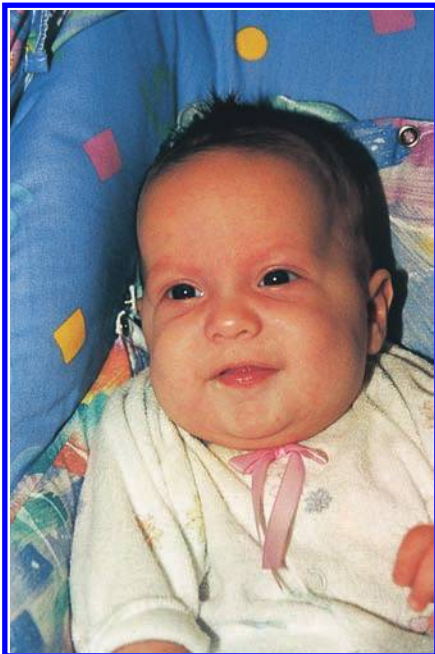


Figure 96.3 SA: The same infant at five months had begun to manifest developmental delay, but appeared alert and happy. Bifrontal diameter was narrow.



Figure 96.5 MC: A nine-month-old infant with far advanced manifestations of the Krabbe disease. The body was stiff throughout and the hands clenched.

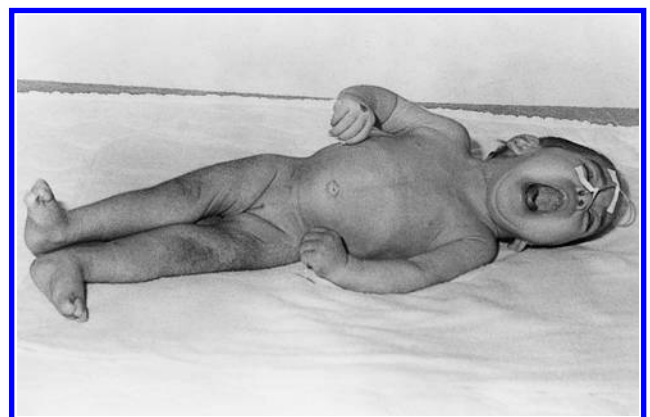


Figure 96.6 This view at nine months illustrates the spasticity, opisthotonus, and clenching of the hands.

clenched and the legs extended. An occasional patient is stiff from birth, and there may be irritability and twitching [14, 15]. Vomiting may be an early symptom [16].

Patients are hypersensitive to sound, light, or touch, and these stimuli set off screaming and rigidity. There may be unexplained fever or convulsive seizures [1, 17]. Some impairment or regression of psychomotor development may be evident early. The level of protein in cerebrospinal fluid (CSF) is elevated at the time of first symptomatology [16], and electrophoresis indicates increase in albumin and decrease in β -globulins. In the second stage of progression

[17] the patient is rigid, in opisthotonos, with the head bent well back. The upper extremities are flexed at the elbows, and the hands are clenched. The lower extremities are usually extended at the hips, knees, and ankles, and they

are adducted so much that they cross. This may ultimately become the patient's constant position. Deep tendon reflexes are diminished. Motor and mental deterioration is rapid. Mild pallor may be seen in the optic disks, and the pupillary response to light may be sluggish. Convulsive seizures may be tonic, clonic, or myoclonic.

The third stage, by 9–12 months [17], is one of decerebrate blindness, deafness, and flaccidity (Figures 96.5 and 96.6). These patients lose all contact with their surroundings and require tube feeding. Cherry red macular spots have been reported by 13–17 months [18]. Death occurs around two years of age, usually from aspiration pneumonia. Frequent vomiting may lead to malnutrition, as well as aspiration and pneumonia. One patient was admitted to hospital at 8 weeks with failure to thrive, feeding problems, and weakness [15]; there were seizures, and deterioration was rapid to death at 15 weeks. Recurrent fever of unknown origin is common. The stiffness of the muscles is always greater in the lower extremities.

These patients often have microcephaly but macrocephaly has been observed [18–21], as has hydrocephalus [22]. They have no hepatosplenomegaly or bony abnormalities. One patient had ichthyosis [20]. Protruding ears have been described as a feature of the disease [23].

Peripheral neuropathy may not be recognized clinically, but the knee jerks may be observed to disappear [1, 19, 24, 25]. A segmental demyelination of the peripheral nerves is seen [26] and nerve conduction velocity is decreased [21, 23]. In one patient, diagnosed prenatally, neurologic examination was normal in the neonatal period, but deep tendon reflexes were absent by 5 weeks [27]. By 7 weeks, peripheral nerve conduction velocity was abnormal. Psychomotor development was normal for two months; weakness of neck muscles was first found at 3 weeks. Elevation of the protein concentration of the CSF may be helpful in suggesting the diagnosis. The electrophoretic pattern of the CSF protein in which albumin and α -globulin are increased, while β - and γ -globulin are decreased, is also seen in metachromatic leukodystrophy.

Nonclassic or late-onset forms of Krabbe galactosylceramide lipidosis have been recognized increasingly since the advent of enzymatic diagnosis [28–33]. Heterogeneity of phenotype has been considerable. Most have presented by ten years of age, but in others neurologic signs developed between ten and 20 years, and one was reported at 39 years of age [29, 34, 35]. In the late infantile group of patients in whom the onset was between six months and three years [36], the manifestations and progression were little different from the classic disease, and death usually ensued within two years of onset. In a second group, in whom the onset was three to eight years [36], the progression was slower, and none had died in the period of follow up, which was as long as seven years. Some were developmentally delayed before the onset of deterioration [31, 32, 37]; some had seizures [38–40]; and two had hemiparesis, progressive in one to tetraplegia

[41]. Onset with ataxia has been observed [31, 32]. Adult patients have been described in whom onset was between 10 and 35 years of age. The CSF protein is abnormal in the late infantile patients, but may be normal or only slightly elevated in juvenile or adult patients [34, 35, 42]. Adult onset patients are being increasingly reported [33, 43] with progressive spastic paraparesis or peripheral neuropathy. Others have had dementia.

In classic Krabbe disease and its variants, neuroimaging usually indicates diffuse cerebral atrophy [44–46]. The scan may be normal early in the disease [47]. Diffuse hypodensity of the white matter has also been described [48]. Plaque-like high intensity T_2 signal has been observed in periventricular and cerebellar white matter in three patients [49].

The electroencephalogram (EEG) is disorganized and slow [14, 19, 20], and there are paroxysmal discharges. There may be asymmetry. The electromyogram (EMG) may be abnormal, and there may be fibrillations [19, 23, 24]. Motor nerve conduction velocity is regularly decreased [19, 23, 25]. In 82 percent of 27 patients, 1 day to eight years old, there was uniform slowing of sensory and motor nerve conduction [50]. The patient may have hyperactive deep tendon reflexes, while electrophysiologic studies indicate a prominent peripheral neuropathy [24]. Among adult patients nerve conduction may be normal [34], or there may be EMG evidence of demyelinating neuropathy [35]. Visual or auditory evoked responses may be abnormal [35]. The former are abnormal early, while the latter abnormalities occur later. In 20 early onset patients, 88 percent had abnormal brain stem auditory evoked potentials (BAEP) and 50 percent abnormal visual evoked potentials (VEP); 65 percent had abnormal EEGs [51]. Palatalmyoclonus has been described in this disease [52].

The neuroanatomic pathology of Krabbe disease is characterized by an extreme hardness or sclerosis of the white matter. Prior to the availability of enzymatic assay, the diagnosis was often established antemortem, by biopsy of brain, which revealed diffuse loss of myelin, astrocytic

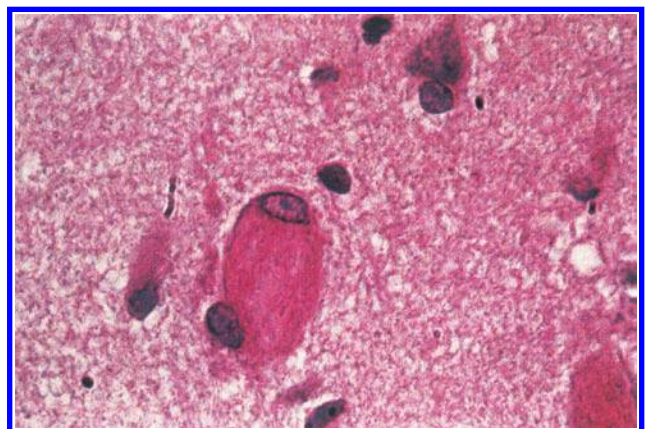


Figure 96.7 The globoid cell that is the hallmark of the Krabbe disease. Section was taken from the brain of MC.

gliosis and the hallmark finding of a massive infiltration with the multinucleated globoid cells (Figure 96.7) in the white matter [1, 19, 53, 54]. These large irregular cells range from 20 to 50 microns in diameter and contain as many as 20 nuclei. The ultrastructure of the globoid cells reveals abnormal tubular crystalloid inclusions [14, 19, 21, 54]. The same inclusions are seen in globoid cells produced in rats, by intracerebral injection of galactocerebroside [55]. These observations suggested that the cells accumulate galactosylceramide, and this has been documented by chemical analysis [2]. Peripheral nerves appear grossly thick and chalk-white [19, 26]. Histologically, there is endoneurial fibrosis and complete loss or thinning of myelin sheaths [25].

GENETICS AND PATHOGENESIS

The disease is transmitted as an autosomal recessive trait [56]. Multiple sibs have been reported with normal parents. In Krabbe's original report there were two sets of siblings [1]. Parental consanguinity has also been observed. There is no ethnic preponderance, and the disorder has been seen throughout the world. In a report from Israel [23] all the patients were Arab. The disease appears to be common in Scandinavia; the incidence in Sweden was calculated to be 1.9 per 100,000 births [56], and in Japan the estimate was one in 100,000–200,000. The parents of patients have been found to have enzyme activity that is distinctly lower than normal and higher than in patients [57]. However, carrier detection is not always reliable, because values in some carriers may overlap the normal range. Prenatal diagnosis of an affected fetus was first reported in 1971 [58]. It is now possible by enzymatic assay of chorionic villus material, as well as amniocytes. It is recommended that enzyme assay be carried out on parents before prenatal diagnosis is undertaken to avoid a false positive in the case of a very low value in a heterozygote. Once the mutation is known, molecular diagnosis may be carried out for heterozygosity and prenatal diagnosis.

The structure of galactosylceramide is shown in Figure 96.1. Cerebrosides are monohexosyl ceramides in which the sugar is glycosidically linked to the C-1 of ceramide. Galactosylceramide is the characteristic cerebroside of myelin and of the central nervous system. The compound is normally degraded to ceramide and galactose by the lysosomal enzyme galactosylceramide β -galactosidase [6]. In patients, the level of activity has been documented to be 5–10 percent normal in brain, liver, spleen, and kidney [6, 7]. The assay is conveniently and reliably performed on leukocytes or cultured fibroblasts [59]. Enzymatic diagnosis with the natural substrate is demanding and should be carried out in an experienced laboratory [57, 60].

A mutant allele has been reported [61] in which the galactosidase activity overlaps that of patients with Krabbe disease. The proband of the first family was a healthy

public health nurse who had volunteered as a control in a study of Krabbe disease. Her leukocyte enzyme activity was consistently lower than 10 percent of control. The presence of this new allelic gene could lead to a misdiagnosis of Krabbe disease, especially *in utero*. The situation could be like that of the Duarte variant for galactose-1-phosphateuridylyltransferase, in which compound variants have been observed who were heterozygous for both the gene for galactosemia and that for the Duarte variant. These findings reinforce the recommendation to establish the enzymatic profile in parents before undertaking a prenatal diagnosis. Enzymatic assay does not distinguish infantile from late onset forms of the disease. Methodology has been developed for dried blood spots in which the product is assayed by tandem mass spectrometry, which permits newborn screening [62].

The twitcher mouse has an autosomally recessively determined deficiency of galactosylceramide β -galactosidase and is an interesting model for Krabbe disease [63]. Other models have been found in West Highland and Cairn terriers, sheep, and monkeys. In the mouse the gene has been mapped to chromosome 12 [64].

The cDNA for the GALC gene contains 3795 base pairs and codes for 669 amino acids [8]. There are 59 and 39 untranslated regions. Expression has been documented by transfer to COS-1 cells.

A rapid test of genomic DNA for the common 502T/del mutation has been developed [13]. In Holland this accounts for 50 percent of mutant alleles [65]. Two other mutations, p.C1538T and p.A1652C, are relatively common in patients of European ancestry [11, 65]. In Israel, homozygosity for c.T1748G (p.I5835) is found in the Druze population, and c.C1582T (p.D528N) in Arab Muslim patients [11]. Among late onset patients c.G809A is relatively common. A polymorphism, c.T1637C, which reduces activity slightly, is found on one allele and a disease-causing mutation, such as c.502T/del or c.G809A on the other in some late onset patients [66]. In 17 Japanese patients six novel mutations were reported [67]. Two were nonsense (p.W115X and p.R204X). They observed that 12del3ins and p.I66M and I289V, which have been found only in Japanese, accounted for 37 mutant alleles, and with p.G270D and p.T652P accounted for 57 percent of mutations in Japanese patients.

The pathogenesis of disease in galactosylceramide lipidosis is not clear. It is an unusual lipid storage disease, in that the stored substrate accumulates only in globoid cells. Storage cannot be demonstrated in lysosomes. The disease in the mouse differs in that inclusions are seen, and the cerebroside accumulates in both kidney and lymphocytes.

In both mouse and man, levels of psychosine were increased in brain and peripheral nerves [68, 69]. This compound, galactosylsphingosine, which differs from the cerebroside in the absence of the fatty acid, is not present in large amounts, but it is essentially absent from normal brain. The terminal galactose is cleaved from this compound, too, by the enzyme that is defective in Krabbe disease. Psychosine is a natural detergent and highly toxic

[70]. Oligodendroglia appear to be selectively destroyed by psychosine formed within them.

TREATMENT

Effective specific treatment has not yet been devised. Bone marrow transplantation has been performed in a few late-onset patients without clear evidence of efficacy [29, 71, 72], although stabilization of some late-onset patients appears to have been accomplished by hematopoietic stem cell transplantation [73]. Among 11 infantile-onset type patients given stem cell transplantation before the onset of symptoms, they were reported [74] to have normal levels of galactocerebroside in blood, progressive myelination and normal cognitive function in most, but some had delayed development. The cloning of the gene and the availability of animal models provide avenues for the study of gene therapy [75].

REFERENCES

- Krabbe K. A new familial infantile form of diffuse brain-sclerosis. *Brain* 1916; **39**: 74.
- Austin JH. Studies in globoid (Krabbe) leukodystrophy. *Neurology* 1969; **19**: 1094.
- Blackwood W, Cummings JN. A histochemical and chemical study of three cases of diffuse cerebral sclerosis. *J Neurol Neurosurg Psychiatry* 1954; **17**: 33.
- Austin J, Lehfeldt D, Maxwell W. Experimental globoid bodies in white matter and chemical analysis in Krabbe's disease. *J Neuropathol Exp Neurol* 1961; **20**: 284.
- Olsson R, Sourander P, Svennerholm L. Experimental studies on the pathogenesis of leukodystrophies. I The effect of intracerebrally injected sphingolipids in the rat brain. *Acta Neuropathol (Berl)* 1966; **6**: 153.
- Suzuki K, Suzuki Y. Globoid cell leukodystrophy (Krabbe's disease): deficiency of galactocerebroside β -galactosidase. *Proc Natl Acad Sci USA* 1970; **66**: 302.
- Austin J, Suzuki K, Armstrong D *et al*. Studies in globoid (Krabbe) leukodystrophy (GLD). V Controlled enzymatic studies in ten human cases. *Arch Neurol* 1970; **23**: 502.
- Chen YQ, Rafi MA, de Gala G, Wenger DA. Cloning and expression of cDNA encoding human galactocerebroside the enzyme deficient in globoid cell leukodystrophy. *Hum Mol Genet* 1993; **2**: 1841.
- Zlotogora J, Chakraborty S, Knowlton RG, Wenger DA. Krabbe disease locus mapped to chromosome 14 by genetic linkage. *Am J Hum Genet* 1990; **47**: 37.
- Cannizzaro LA, Chen YQ, Rafi MA *et al*. Regional mapping of the human galactocerebroside gene (GALC) to 14q31 by *in situ* hybridization. *Cytogenet Cell Genet* 1994; **66**: 244.
- Wenger DA, Rafi MA, Luzi P. Molecular genetics of Krabbe disease (globoid cell leukodystrophy): diagnostic and clinical aspects. *Hum Mutat* 1997; **10**: 268.
- Rafi MA, Luzi P, Chen YQ, Wenger DA. A large deletion together with a point mutation in the GALC gene is a common mutant allele in patients with infantile Krabbe disease. *Hum Mol Genet* 1995; **4**: 1285.
- Luzi P, Rafi MA, Wenger DA. Characterization of the large deletion in the GALC gene found in patients with Krabbe disease. *Hum Mol Genet* 1995; **4**: 2335.
- Schochet SS Jr, Hardman JM, Lampert PW, Earle KM. Krabbe's disease (globoid leukodystrophy): electron microscopic observations. *Arch Pathol* 1969; **88**: 305.
- Clarke JTR, Ozere RL, Krause VW. Early infantile variant of globoid cell leukodystrophy with lung involvement. *Arch Dis Child* 1981; **8**: 640.
- Hagberg B, Sourander P, Svennerholm L. Diagnosis of Krabbe's infantile leukodystrophy. *J Neurol Neurosurg Psychiatry* 1963; **26**: 195.
- Hagberg B. The clinical diagnosis of Krabbe's infantile leukodystrophy. *Acta Paediatr Scand* 1963; **52**: 213.
- Hofman KJ, Naidu S, Moser HW *et al*. Cherry red spot in association with galactosylceramide-beta-galactosidase deficiency. *J Inher Metab Dis* 1987; **10**: 273.
- Suzuki K, Grover WD. Krabbe's leukodystrophy (globoid cell leukodystrophy): an ultrastructural study. *Arch Neurol* 1970; **22**: 385.
- Nelson E, Aurebeck G, Osterberg K *et al*. Ultrastructural and chemical studies on Krabbe's disease. *J Neuropathol Exp Neurol* 1963; **22**: 414.
- Yunis EJ, Lee RE. The ultrastructure of globoid (Krabbe) leukodystrophy. *Lab Invest* 1969; **21**: 415.
- Laxdal T, Hallgrimsson K. Krabbe's globoid cell leukodystrophy with hydrocephalus. *Arch Dis Child* 1974; **49**: 23.
- Zlotogora J, Chakraborty S, Knowlton RG *et al*. Krabbe disease and protruding ears. *Am J Med Genet* 1987; **28**: 759.
- Moosa A. Peripheral neuropathy and ichthyosis in Krabbe's leukodystrophy. *Arch Dis Child* 1971; **46**: 112.
- Hogan GR, Gutmann L, Chou SM. The peripheral neuropathy of Krabbe's (globoid) leukodystrophy. *Neurology* 1969; **19**: 1094.
- Matsuyama H, Minoshima I, Watanabe I. An autopsy case of leukodystrophy of Krabbe type. *Acta Pathol Jpn* 1963; **13**: 195.
- Lieberman JS, Oshtory M, Taylor RG, Dreyfus PM. Perinatal neuropathy as an early manifestation of Krabbe's disease. *Arch Neurol* 1980; **37**: 446.
- Lyon G, Hagberg B, Evrard PH *et al*. Symptomatology of late onset Krabbe's leukodystrophy: the European experience. *Dev Neurosci* 1991; **13**: 240.
- Kolodny EH, Raghavan S, Krivit W. Late onset Krabbe disease (globoid cell leukodystrophy): clinical and biochemical features of 15 cases. *Dev Neurosci* 1991; **13**: 322.
- Fiumara A, Pavone L, Siciliano L *et al*. Late-onset globoid cell leukodystrophy: report on seven new patients. *Childs Nerv Syst* 1990; **6**: 194.
- Crome L, Hanefeld F, Patrick D, Wilson J. Late onset globoid cell leukodystrophy. *Brain* 1973; **96**: 84.
- Hanfeld F, Wilson J, Crome L. Die juvenile form der globoidzell-leukodystrophie. *Monatsschr Kinderheilkd* 1973; **121**: 293.

33. Henderson RD, MacMillan JC, Bradfield JM. Adult onset Krabbe disease may mimic motor neurone disease. *J Clin Neurosci* 2003; **10**: 638.
34. Grewal RP, Petronas N, Barton NW. Late-onset globoid cell leukodystrophy. *J Neurol Neurosurg Psychiatry* 1991; **54**: 1011.
35. Verdru P, Lammens M, Dom R *et al*. Globoid cell leukodystrophy: a family with both late-infantile and adult type. *Neurology* 1991; **41**: 1382.
36. Loonen MCB, Van Diggelen OP, Janse HC *et al*. Late-onset globoid cell leukodystrophy (Krabbe's disease). Clinical and genetic delineation of two forms and their relation to the early infantile form. *Neuropediatrics* 1985; **16**: 137.
37. Kolodny EH, Adams RD, Haller JS *et al*. Late-onset globoid cell leukodystrophy. *Ann Neurol* 1980; **8**: 219.
38. Malone MJ, Szoke MC, Looney GL. Globoid leukodystrophy. I Clinical and enzymatic studies. *Arch Neurol* 1975; **32**: 606.
39. Vos AJM, Joosten EMG, Gabreëls-Festem AAWM, Gabreëls FJM. An atypical case of infantile globoid cell leukodystrophy. *Neuropediatrics* 1983; **14**: 110.
40. Goebel HH, Harzer K, Ernst JP *et al*. Late-onset globoid leukodystrophy: unusual ultrastructural pathology and subtotal beta-galactocerebrosidase deficiency. *J Child Neurol* 1990; **5**: 299.
41. Rolando S, Cremonese M, Leonardi A. Late onset globoid leukodystrophy: unusual clinical and CSF findings. *Ital J Neurol Sci* 1990; **11**: 57.
42. Phelps M, Aicardi J, Vanier MT. Late-onset Krabbe's leukodystrophy. A report of four cases. *J Neurol Neurosurg Psychiatry* 1991; **54**: 293.
43. Satoh J-I, Tokumoto H, Kurohara K *et al*. Adult-onset Krabbe disease with homozygous T1853C mutation in the galactocerebrosidase gene. Unusual MRI findings of corticospinal tract demyelination. *Neurology* 1997; **49**: 1392.
44. Demareel P, Wilms G, Verdru P *et al*. Findings in globoid cell leukodystrophy. *Neuroradiology* 1990; **32**: 520.
45. Lane B, Carroll BA, Pedley TA. Computerized cranial tomography in cerebral diseases of white matter. *Neurology* 1978; **28**: 534.
46. Heinz ER, Drayer BP, Haenggeli CA *et al*. Computed tomography in white matter disease. *Radiology* 1979; **130**: 371.
47. Barnes DM, Enzmann DR. The evolution of white matter disease as seen on computed tomography. *Radiology* 1981; **138**: 379.
48. Ieshima A, Eda S, Matsui A *et al*. Computed tomography in Krabbe's disease: comparison with neuropathology. *Neuroradiology* 1983; **25**: 323.
49. Sasaki M, Sakuragawa N, Takashima S *et al*. MRI and CT findings in Krabbe disease. *Pediatr Neurol* 1991; **7**: 283.
50. Siddiqi ZA, Sanders DB, Massey JM. Peripheral neuropathy in Krabbe disease: electrodiagnostic findings. *Neurology* 2006; **67**: 263.
51. Husain AM, Altuwaijri M, Aldosari M. Krabbe disease: neurophysiologic studies and MRI correlations. *Neurology* 2004; **63**: 617.
52. Yamanouchi H, Kasai H, Sakuragawa N, Kurokawa T. Palatal myoclonus in Krabbe disease. *Brain Dev* 1991; **13**: 355.
53. Wallace BJ, Aronson SM, Volk BW. Histochemical and biochemical studies of globoid cell leukodystrophy (Krabbe's disease). *J Neurochem* 1963; **11**: 367.
54. Yunis EJ, Lee RE. Further observations on the fine structure of globoid leukodystrophy: peripheral neuropathy and optic nerve involvement. *Hum Pathol* 1972; **3**: 371.
55. Austin JH, Lehfeldt D. Studies in globoid (Krabbe) leukodystrophy. III Significance of experimentally produced globoid-like elements in rat white matter and spleen. *J Neuropathol Exp Neurol* 1965; **24**: 265.
56. Hagberg B, Kollberg H, Sourander P, Akesson HO. Infantile globoid cell leukodystrophy (Krabbe's disease). Clinical and genetic studies of 11 Swedish cases 1953–1967. *Neuropediatrics* 1969; **1**: 74.
57. Wenger DA, Sattler M, Clark C, McKelvey H. An improved method for the identification of patients and carriers of Krabbe's disease. *Clin Chim Acta* 1974; **56**: 199.
58. Suzuki K, Schneider EL, Epstein CJ. *In utero* diagnosis of globoid cell leukodystrophy (Krabbe's disease). *J Pediatr* 1976; **88**: 76.
59. Suzuki Y, Suzuki K. Krabbe's globoid cell leukodystrophy: deficiency of galactocerebrosidase in serum leukocytes and fibroblasts. *Science* 1971; **171**: 73.
60. Vanier MT, Svennerholm L, Mansson JE *et al*. Prenatal diagnosis of Krabbe disease. *Clin Genet* 1981; **20**: 79.
61. Wenger DA, Riccardi VM. Possible misdiagnosis of Krabbe's disease. *J Pediatr* 1976; **88**: 76.
62. Li Y, Scott CR, Chamoles NA *et al*. Direct multiplex assay of lysosomal enzymes in dried blood spots for newborn screening. *Clin Chem* 2004; **50**: 1785.
63. Suzuki K, Suzuki K. The twitcher mouse: a model of human globoid cell leukodystrophy (Krabbe's disease). *Am J Pathol* 1983; **111**: 394.
64. Sweet H. Twitcher is on Ch 12. *Mouse Newslett* 1986; **75**: 30.
65. Kleijer WJ, Keulemans JLM, van der Kraan M *et al*. Prevalent mutations in the GALC gene of patients with Krabbe disease of Dutch and other European origin. *J Inher Metab Dis* 1997; **20**: 587.
66. Harzer K, Knoblich R, Rolfs A *et al*. Residual galactosylsphingosine (psychosine) beta-galactosidase activities and associated GALC mutations in late and very late onset Krabbe disease. *Clin Chim Acta* 2002; **317**: 77.
67. Xu C, Sakai N, Taniike M *et al*. Six novel mutations detected in the GALC gene in 17 Japanese patients with Krabbe disease, and new genotype-phenotype correlation. *J Hum Genet* 2006; **51**: 548.
68. Scaravilli F, Jacobs JM, Teixeira F. Quantitative and experimental studies on the twitcher mouse. In: Baumann N (ed.). *Neurological Mutations Affecting Myelination*. Amsterdam: Elsevier, 1980: 115.
69. Miyatake T, Suzuki K. Globoid cell leukodystrophy: additional deficiency of psychosine galactosidase. *Biochem Biophys Res Commun* 1972; **48**: 538.
70. Suzuki K, Tanaka H, Suzuki K. Studies on the pathogenesis of Krabbe's leukodystrophy. Cellular reaction of the brain to exogenous galactosylsphingosine monogalactosyl diglyceride and lactosylceramide. In: Volk BW, Schneck L (eds). *Current Trends in Sphingolipidoses and Allied Disorders*. New York: Plenum Press, 1976: 99.

71. Choi KG, Sung JH, Clark HB, Krivit W. Pathology of adult-onset globoid cell leukodystrophy (GLD). *J Neuropathol Exp Neurol* 1991; **50**: 336.
72. Krivit W, Whitley CB, Chang P-N *et al*. Lysosomal storage diseases treated by bone marrow transplantation: review of 21 patients. In: Johnson FL, Pochedly C (eds). *Bone Marrow Transplantation in Children*. New York: Raven, 1990: 261.
73. Krivit W, Shapiro EG, Peters C *et al*. Hematopoietic stem-cell transplantation in globoid-cell leukodystrophy. *N Engl J Med* 1998; **338**: 1119.
74. Escolar ML, PoeMD, Provenzale JM *et al*. Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease. *N Engl J Med* 2005; **19**: 352.
75. Wenger DA, Rafi MA, Luzi P *et al*. Krabbe disease: genetic aspects and progress toward therapy. *Mol Genet Metab* 2000; **70**: 1.

Wolman disease/cholesteryl ester storage disease

Introduction	733	Treatment	737
Clinical abnormalities	733	References	737
Genetics and pathogenesis	737		

MAJOR PHENOTYPIC EXPRESSION

Vomiting, diarrhea, failure to thrive, abdominal distension, hepatosplenomegaly, adrenal calcification, vacuolated peripheral lymphocytes and foam cells in the marrow, storage of cholesterylesters and triglycerides, and deficiency of lysosomal acid lipase. The cholesteryl ester storage disease phenotype is of a later onset progressive hepatic disease that may be progressive to cirrhosis.

INTRODUCTION

Wolman and colleagues [1] reported first one, then two more siblings in the same family, in whom the accumulation of cholesterol and triglycerides was associated with abdominal distension, hepatosplenomegaly, and calcification of the adrenals. Death occurred within the first three months of life. The molecular defect in this disease is the lysosomal acid lipase (EC 3.1.1.13) [2]. This lipase, first demonstrated to be defective in liver and spleen, is a 46 kDa glycoprotein

active on both triglycerides and cholesteryl esters (Figure 97.1). The enzyme is also defective in cholesteryl ester storage disease. The two diseases are allelic, caused by mutations at the *LIPA* locus on chromosome 10q23.2-q23.3 [3]. In general, the mutations in patients with Wolman disease are major alterations that lead to absence of enzyme activity [4–6]. Most patients with cholesteryl ester storage disease have at least one copy of a single mutant allele, a G934A mutation at the exon 8 splice junction, which leads to exon skipping and the loss of codons 254–277 [7, 8].

CLINICAL ABNORMALITIES

Symptoms of Wolman disease begin in the early weeks of life, and most patients have died by six months of age; survival as long as 14 months has been observed [2]. Infants appear normal for 2–7 weeks; then they develop diarrhea and vomiting [1, 9–11]. This presentation is sufficiently nonspecific that patients are usually thought at first to have gastroenteritis. Stools remain watery and green, and soon failure to thrive is evident (Figure 97.2). In a few infants, loose watery stools occur in the first weeks of life [12, 13]. As symptoms persist, an intestinal or malabsorption etiology is generally sought. The abdomen regularly becomes impressively distended (Figures 97.3 and 97.4). This may lead to laparotomy in a search for intestinal obstruction [14], or for other reasons [9] (Figure 97.5). The diagnosis of a lipid storage disease will usually be evident on laparotomy

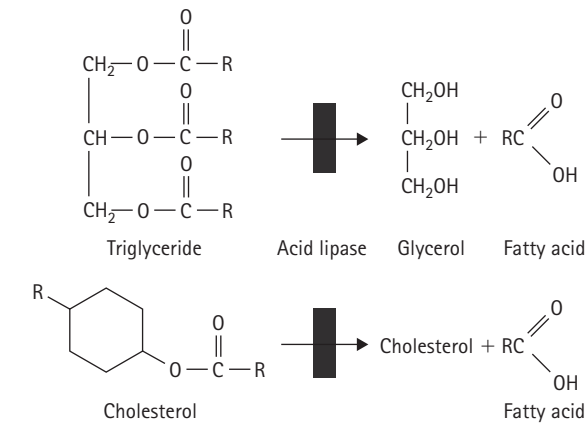


Figure 97.1 Schematic view of acid lipase, the site of the defect in Wolman and cholesteryl ester storage diseases. The enzyme catalyzes the release of free fatty acids from triglycerides and from cholesteryl esters.

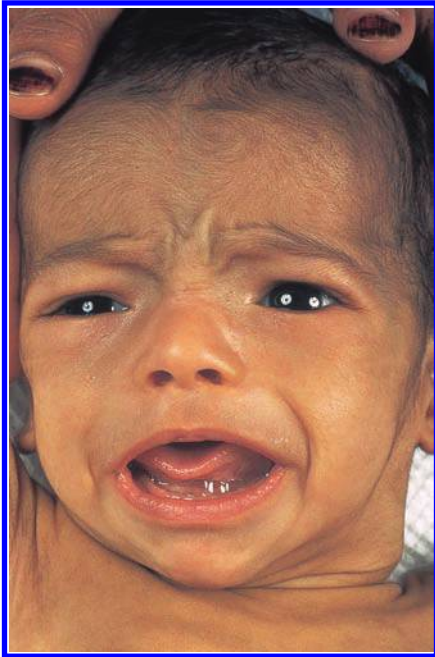


Figure 97.2 SIIS: A three-month-old boy with Wolman disease. The emaciation is evident in the face.



Figure 97.4 IMIS: A two-month-old boy, a previous sibling of the boy in Figures 97.1 and 97.2, also with Wolman disease. He was seriously ill, endotracheally intubated, and died 12 days later. He developed watery, green diarrhea and vomiting at 3 weeks of age and was admitted to hospital for abdominal distress at 23 days.



Figure 97.3 The distended abdomen and prominent venous pattern of the same patient. The liver was enlarged. Enzyme assay of cultured fibroblasts revealed less than 5 percent of control activity.



Figure 97.5 Abdominal distension remained massive in this patient following surgery for what had been thought to be intestinal obstruction, and there was dehiscence of the wound. Grossly enlarged and yellow lymph nodes were noted at surgery. Histologic examination revealed lipid storage.

because of the appearance of the liver and spleen. Biopsy will confirm the presence of lipid storage. Not all patients come to laparotomy; thus other clues to the diagnosis must be sought. Affected patients appear wasted and severely ill (Figures 97.2 and 97.4) [9, 10, 14]. Some patients have jaundice [10, 11, 15] and some, a low grade fever [1, 10]. There is impressive, massive enlargement of the liver and spleen [1, 12–14]. Hepatosplenomegaly may be evident as early as the fourth day of life [12] and may be massive.

Calcification of the adrenals is a hallmark feature of this disease [16]. In an infant with the usual clinical manifestations, it should lead to the diagnosis. Calcification

may be seen on plain roentgenogram of the abdomen, as fine-stippled or discrete, punctate calcification [9, 10]. However, it may be readily missed on routine roentgenograms, especially in the presence of ascites. It is no accident that the most frequently reproduced illustration is of a roentgenogram of adrenals following their removal at autopsy [10]. The calcifications are diffuse, and follow the

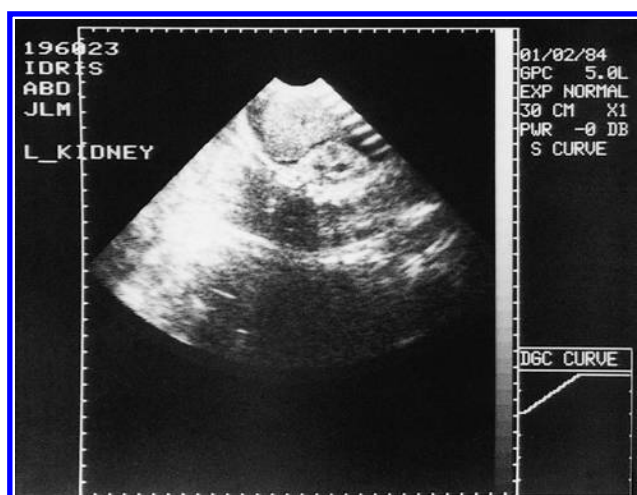


Figure 97.6 Ultrasound of the left kidney shows a dark acoustic shadow resulting from the calcification in the adrenal.

outline of the glands. This appearance distinguishes these adrenal calcifications from those of adrenal hemorrhage or a neuroblastoma. The earliest appearance may be of enlarged adrenals, which may displace the kidney downward or flatten the superior pole, without deforming the caliceal system or interfering with renal function. Over the next few months of life the adrenals shrink and become increasingly calcified. The calcifications may be found on ultrasonographic examination (Figure 97.6), in which a dark acoustic shadow is evident. The best way to visualize calcified adrenals is with a computed tomography (CT) scan (Figures 97.7 and 97.8); various cuts permit an estimation of the size of the adrenals, and the dense calcification is readily evident.

Other roentgenographic features of the disease include



Figure 97.7 Computed tomography scan of the abdomen reveals the calcifications in the left adrenal and enlargement of the right adrenal.

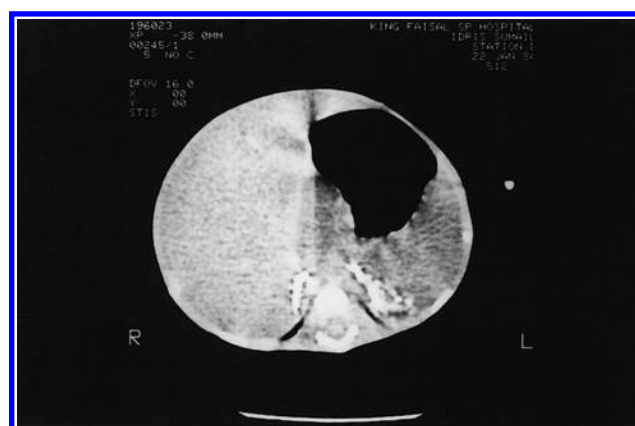


Figure 97.8 In this view, the computed tomography scan clearly shows both adrenals to be densely calcified.

the hepatosplenomegaly and/or ascites. The bones are usually hypodense, and there may be a wide marrow cavity and thin cortex, or poor modeling [10].

Anemia is a prominent early feature of the disease [1, 9, 10], usually evident by 6 weeks. It worsens progressively and may require transfusion. Acanthocytosis has been reported [17]. Thrombocytopenia is not a feature of the disease. Vacuolated lymphocytes or granulocytes (Figure 97.9) may be found in the peripheral blood. The vacuoles are both intracytoplasmic and intranuclear. In many patients the initial clinical impression is first confirmed by the aspiration of lipid-laden histiocytes from the bone marrow (Figure 97.10). These foam cells are quite similar to those found in Niemann-Pick disease [18], and a number of patients reported as Niemann-Pick disease with adrenal calcifications were probably early examples of Wolman disease. Rarely, phagocytosis of erythrocytes by these cells may be seen [9]. These large, pale, foamy cells may be present in the marrow as early as 40 days. Later, they are present in large numbers and may even be seen in the

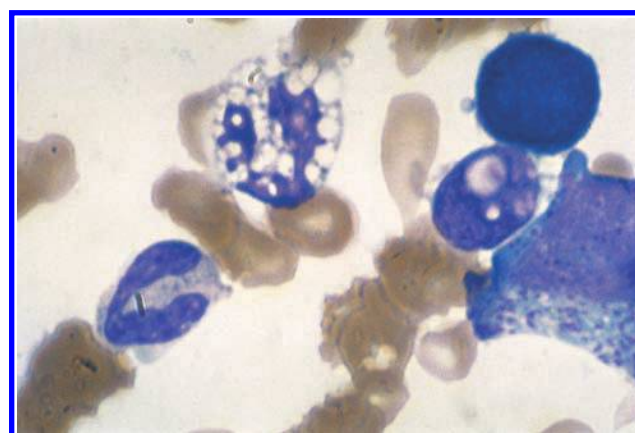


Figure 97.9 Vacuolation in a peripheral blood granulocyte.

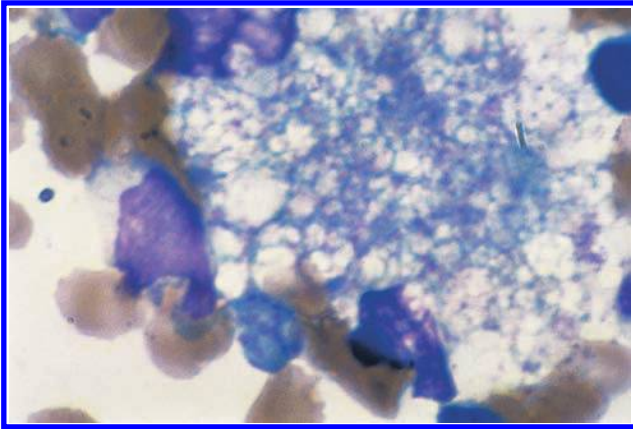


Figure 97.10 Large foamy histiocyte from an aspirate of bone marrow.

peripheral blood [1]. Electronmicroscopic examination may reveal vacuolation and granular inclusions in circulating granulocytes; vacuoles may be seen on light microscopy.

Psychomotor development appears delayed or to deteriorate, but these patients are so ill that it is difficult to assess whether or not the nervous system is abnormal. Neurologic examination may be normal. Patients are often described as bright and alert but weak [9]. Deep tendon reflexes may be hypoactive or brisk [10], and the plantar response may be extensor, but this may be normal at this age. In one patient [11] deep tendon reflexes were exaggerated, and there was ankle clonus, as well as opisthotonos. The electroencephalogram is usually normal [10, 11, 18].

Plasma cholesterol and lipids may be low or normal [9, 17], or the serum may be hyperlipemic in the fasting state [9], and levels of lipids may be elevated. The lipid is largely triglyceride. The erythrocyte sedimentation rate may be elevated. Liver function tests are usually abnormal [2]. Hypoglycemia may occur as hepatic function deteriorates. Malabsorption can be shown using ^{131}I -labeled triolein [18] or unlabeled fat [12] to demonstrate impaired absorption of fat. Administration of adrenocorticotrophic hormone (ACTH) may reveal diminished responsiveness of the adrenals [12].

Pathologic examination [1, 10, 14, 19] of material obtained at biopsy or autopsy showed the liver to be yellow or yellow-tan and greasy on its cut surface. Hepatic architecture is distorted, so that only the portal spaces may be recognizable. Foamy macrophages or Kupffer cells are scattered amid large vacuolated hepatocytes. By the time of death periportal fibrosis is the rule, and there may be frank portal cirrhosis [10, 12, 20]. Electron microscopic examination discloses well-defined fat droplets bound by a trilaminated membrane and, with the exception of hepatocytes, slender crystals [20]. Most fat droplets are within lysosomes [17]. The endoplasmic reticulum may be distended [21]. The adrenals are large and pale or bright yellow. Calcifications may be felt as gritty on cutting. On

section it is the outer cortex that is yellow; the central zone is gray. The histologic architecture is preserved, but the cells are large, vacuolated, and swollen [9, 10, 14]. Foam cells contain sudanophilic material; some contain birefringent crystals, and occasionally the Maltese crosses typical of cholesterol [12, 20]. Some foam cells become necrotic and it is in these areas that calcification is prominent. It may be condensed in dense crystalline lumps [9]. There may be extensive fibrosis.

The small bowel may be yellow, thickened, and dilated [14, 20], and changes are most marked in the proximal small bowel. Pneumatosis has been described in the colon [14]. Infiltration of the small intestine by foamy histiocytes is extensive, and the mucosal cells are also foamy. These changes appear to account for the malabsorption [14, 21]. In addition, there is infiltration of the ganglion cells of the intestine [14], which may be related to the distension that is so characteristic of these patients. There may also be ileus resulting from potassium losses caused by the chronic diarrhea. The spleen is grossly enlarged, and spleen and lymph nodes largely comprise large, foamy, vacuolated cells. Clear-cut evidence of storage of lipid in neurons of the brain has been reported [14, 22–24]. Swollen glial cells and histiocytes have also been observed. There may be a decrease in the numbers of neurons and impaired myelination [10]. Gliosis of the white matter has been reported [23, 24], but this apparent leukodystrophy may be artifactual [25]. Electronmicroscopic examination has documented extensive accumulation of lipid throughout the central and peripheral nervous system. In the brain, oligodendrocytes were the major sites of storage.

Cholesteryl ester storage disease

Deficiency of the same lysosomal acid lipase that is defective in Wolman disease is found in cholesteryl ester storage disease [26]. Patients with this disorder have a much more indolent disorder which may present with otherwise asymptomatic hepatomegaly or hepatosplenomegaly in childhood or adulthood [27–33]. Massive splenomegaly and a splenic abscess were reported in one patient [32]. Recurrent abdominal pain has occurred in some patients, and some have had recurrent epistaxis or intestinal bleeding. There may be evidence of cirrhosis on biopsy. Esophageal varices have occasionally been observed [31, 34, 35]. Acute or chronic hepatic failure has been reported in a few patients [35, 36]. Some are icteric. Clotting factors, including prothrombin and factor V, may be reduced. Some patients have hyperlipemia and elevation of the plasma concentration of cholesterol. Pulmonary hypertension has been reported as a complication, leading to death at 18 years [36]. Hyperlipoproteinemia type IIb is commonly encountered, and some patients have xanthelasma. There may be impressive premature atherosclerosis.

The reduction in activity of the acid lipase is 50- to 100-fold [37] – severely depressed but much less so than

in Wolman disease. On the other hand, in most assays the difference in activity seen in cholesteryl ester disease is not appreciably different from that of Wolman disease, and certainly not enough to account for the differences in phenotype [26, 38]. However, this is also the case in most attempts to study genetic heterogeneity by enzyme assay in lysates of cells or tissues. Normal amounts of cross-reacting material (CRM) have been found in fibroblasts.

Histologically, the macrophages of the liver are full of cholesteryl esters. A patient was reported to have sea blue histiocytes in the marrow [39].

Patients have been described [40] with similar biochemical and histological findings as those in Wolman disease, but a much more benign course. A considerable heterogeneity and a spectrum of defects in this enzyme is increasingly evident.

GENETICS AND PATHOGENESIS

Chemical analysis of tissues in both Wolman and cholesteryl ester storage diseases reveals increased quantities of cholesteryl esters and triglycerides [9, 10, 41, 42]. This may be readily demonstrated by thin layer chromatography. A high performance liquid chromatography (HPLC) method for the quantification of lipids is useful in the differentiation of Wolman disease, Niemann-Pick disease, and Gaucher disease [43]. It may be used with fibroblasts, lymphocytes, or leukocytes, as well as tissue samples. Lipid analysis has most commonly been reported of liver and spleen, where the triglyceride content may be as much as 10 and 350 times the normal value, and the total cholesterol content is always increased [42]. An eight-fold elevation has been reported in adrenal [12]. Storage of cholesteryl ester has also been documented in fibroblasts [41]. Unusual oxygenated steryl esters such as those of 7- α -hydroxycholesterol have been found in tissues [42].

The defective activity in the acid lipase is consistent with the accumulation of these lipids in tissues. The enzymatic defect is demonstrable in a wide variety of tissues [2, 44], including leukocytes [45–47] and cultured fibroblasts [26, 38, 48]. Lysosomal acid lipase may be separated electrophoretically into three isozymes: A, B, and C. It is the A isozyme that is defective in Wolman and cholesteryl ester storage diseases [26, 49]. Immunochemical studies using antibodies against normal acid lipase revealed cross-reacting material in fibroblasts of patients with both diseases [37]. The amounts of CRM were at the level found in normal cells, while enzyme activity in Wolman disease was reduced 200-fold.

Wolman disease and cholesteryl ester storage disease are caused by allelic recessive genes at the same locus on chromosome 10 [50], causing deficiency of lysosomal acid lipase [34]. Multiple affected siblings of normal parents have been reported in a number of families [11, 51], as has consanguinity [1, 11, 52–54]. Heterozygosity can be detected by assay of acid lipase in leukocytes or

cultured fibroblasts [20, 36, 45–47, 55–57]. Levels are about 50 percent of normal. Prenatal diagnosis has been accomplished in Wolman disease by demonstration of the deficiency of acid lipase in cultured amniocytes [58]. In a family in which the mutation is known, DNA diagnosis may be employed for heterozygote detection and prenatal diagnosis.

The gene for lysosomal acid lipase has been cloned [60] and localized to chromosome 10q23.3 [3]. The gene has been sequenced and contains 10 exons. A number of mutations have been identified [3, 7, 59–62]. The common G934A mutation in cholesteryl ester storage disease leads to a truncated protein missing 24 amino acids [7, 8]. Patients nevertheless have had a variety of levels of enzyme activity. The cholesteryl ester disease phenotype has also been seen in patients with the common mutation in compound with mutations otherwise found in Wolman disease, such as L179P [4]. The G934A mutation has not, however, been found in patients with the Wolman phenotype.

In the first patient with Wolman disease in whom mutations were identified, L179P was in compound with a frameshift mutation at nucleotide 634 (insT) causing a premature stop (Fs178) [4]. A majority of patients with Wolman disease have been homozygotes, and many had truncating mutations [8, 63, 64]. The common exon 8 splice site mutation at-1 was found to yield 3 percent of correctly spliced mRNA and a full-length enzyme [8]. On the other hand, sibs with Wolman disease homozygous for a splice site mutation at the same donor site had no correctly spliced mRNA and no enzyme activity.

TREATMENT

There is no recognized treatment for Wolman disease. The use of HMG CoA reductase inhibitors to reduce cholesterol biosynthesis and apolipoprotein B generation appears prudent in cholesteryl ester storage disease [8, 65]. Hepatic transplantation has been employed in hepatic failure [66]. Despite bone marrow transplantation and engraftment, one patient died of pulmonary dysfunction, and three others were failures despite successful engrafting in two [67]. However, success has been reported [68].

REFERENCES

1. Wolman M, Sterk W, Gatt S, Frenkel M. Primary family xanthomatosis with involvement and calcification of the adrenals. Report of two more cases in siblings of a previously described infant. *Pediatrics* 1961; **28**: 742.
2. Patrick AD, Lake BD. Deficiency of an acid lipase in Wolman's disease. *Nature* 1969; **222**: 1067.
3. Anderson RA, Rao N, Byrum RS et al. *In situ* localization of the genetic locus encoding the lysosomal acid lipase/cholesteryl esterase (LIPA) deficient in Wolman disease to chromosome 10q232-233. *Genomics* 1993; **15**: 245.

4. Anderson RA, Byrum RS, Coates PM, Sando GN. Mutations at the lysosomal acid cholesteryl ester hydrolase gene locus in Wolman disease. *Proc Natl Acad Sci USA* 1994; **91**: 2718.
5. Mayatepek E, Seedorf U, Wiebusch H *et al*. Fatal genetic defect causing Wolman disease. *J Inherit Metab Dis* 1999; **22**: 93.
6. Seedorf U, Guardamagna O, Strobl W *et al*. Mutation report: Wolman disease. *Hum Genet* 1999; **105**: 337.
7. Klima H, Ullrich K, Aslanidis C *et al*. A splice junction mutation causes deletion of a 72-base exon from the mRNA for lysosomal acid lipase in a patient with cholesteryl ester storage disease. *J Clin Invest* 1993; **92**: 2713.
8. Aslanidis C, Ries S, Fehringer P *et al*. Genetic and biochemical evidence that CESD and Wolman disease are distinguished by residual lysosomal acid lipase activity. *Genomics* 1996; **33**: 85.
9. Marshall WC, Ockenden BG, Fosbrooke AS, Cumings JN. Wolman's disease. A rare lipidosis with adrenal calcification. *Arch Dis Child* 1969; **44**: 331.
10. Crocker AC, Vawter GF, Neuhauser EBD, Rosowsky A. Wolman's disease: three new patients with a recently described lipidosis. *Pediatrics* 1965; **35**: 627.
11. Konno T, Fujii M, Watanuki T, Koizumi K. Wolman's disease: the first case in Japan. *Tohoku J Exp Med* 1966; **90**: 375.
12. Lough J, Fawcett JF, Wiegensberg B. Wolman's disease. An electron microscopic histochemical and biochemical study. *Arch Pathol* 1970; **89**: 103.
13. Marks M, Marcus AJ. Wolman's disease. *Can Med Assoc J* 1968; **99**: 232.
14. Kahana D, Berant M, Wolman M. Primary familial xanthomatosis with adrenal involvement (Wolman's disease). Report of a further case with nervous system involvement and pathogenetic considerations. *Pediatrics* 1968; **42**: 70.
15. Kamalian N, Dudley AW, Beroukhim F. Wolman's disease with jaundice and subarachnoid hemorrhage. *Am J Dis Child* 1973; **126**: 671.
16. Abramov A, Schorr S, Wolman M. Generalized xanthomatosis with calcified adrenals. *Am J Dis Child* 1956; **91**: 282.
17. Eto Y, Kitagawa T. Wolman's disease with hypolipoproteinemia and acanthocytosis: clinical and biochemical observations. *J Pediatr* 1970; **77**: 862.
18. Neuhauser BD, Kirkpatrick JA, Wientraub B. Wolman's disease: a new lipidosis. *Ann Radiol* 1965; **8**: 175.
19. Wallis K, Gross M, Kohn R, Zaidman J. A case of Wolman's disease. *Helv Paediatr Acta* 1971; **26**: 98.
20. Schaub J, Janka GE, Christomanou H *et al*. Wolman's disease: clinical biochemical and ultrastructural studies in an unusual case without striking adrenal calcification. *Eur J Pediatr* 1980; **135**: 45.
21. Kamoshita S, Landing BH. Distribution of lesions in myenteric plexus and gastrointestinal mucosa in lipidoses and other neurological disorders of children. *Am J Clin Pathol* 1968; **49**: 312.
22. Wolman M. Involvement of nervous tissue in primary familial xanthomatosis with adrenal calcification. *Pathol Eur* 1968; **3**: 259.
23. Guazzi GC, Martin JJ, Philippart M *et al*. Wolman's disease. *Eur Neurol* 1968; **1**: 334.
24. Guazzi GC, Martin JJ, Philippart M *et al*. Wolman's disease: distribution and significance of the central nervous system lesions. *Pathol Eur* 1968; **3**: 266.
25. Byrd III JC, Powers JM. Wolman's disease: ultrastructural evidence of lipid accumulation in central and peripheral nervous systems. *Acta Neuropathol* 1979; **45**: 37.
26. Cortner JA, Coates PM, Swoboda E, Schnatz JD. Genetic variation of lysosomal acid lipase. *Pediatr Res* 1976; **10**: 927.
27. Frederickson DS. Newly recognized disorders of cholesterol metabolism. *Ann Intern Med* 1963; **58**: 718.
28. Frederickson DS, Sloan HR, Ferran VJ, Demosky SJ Jr. Cholesteryl ester storage disease: a most unusual manifestation of deficiency of two lysosomal enzyme activities. *Trans Assoc Am Physicians* 1972; **85**: 109.
29. Lageron A, Caroli J, Stralin H, Barbier P. Polycorie cholestérolique de l'adulte. 1 Etude clinique électronique histochimique. *Presse Méd (Paris)* 1967; **75**: 2785.
30. Partin JC, Schubert WK. Small intestinal mucosa in cholesterol ester storage disease: a light and electron microscope study. *Gastroenterology* 1969; **57**: 542.
31. Schiff L, Schubert WK, McAdams AJ *et al*. Hepatic cholesterol ester storage disease a familial disorder. 1 Clinical aspects. *Am J Med* 1968; **44**: 538.
32. Elleder M, Ledvinova J, Cieslar P, Kuhn R. Subclinical course of cholesterol ester storage disease diagnosed in adulthood. Report on two cases with remarks on the nature of the liver storage process. *Virchows Arch [A]* 1990; **416**: 357.
33. Edelstein RA, Filling K, Pentschev P *et al*. Cholesteryl ester storage disease: a patient with massive splenomegaly and splenic abscess. *Am J Gastroenterol* 1988; **83**: 687.
34. Wolf H, Hug G, Michaelis R, Nolte K. Seltene angeborene Erkrankung mit Cholesterinester-Speicherung in der Leber. *Helv Paediatr Acta* 1974; **29**: 105.
35. Beaudet AL, Ferry GD, Nichols BL, Rosenberg HS. Cholesterol ester storage disease: clinical biochemical and pathological studies. *J Pediatr* 1977; **90**: 910.
36. Cagle PT, Ferry GD, Beaudet AL, Hawkins EP. Clinopathologic conference: pulmonary hypertension in an 18-year-old girl with cholesteryl ester storage disease (CESD). *Am J Med Genet* 1986; **24**: 711.
37. Burton BK, Reed SP. Acid lipase cross-reacting material in Wolman disease and cholesterol ester storage disease. *Am J Hum Genet* 1981; **33**: 203.
38. Guy GJ, Butterworth J. Acid esterase activity in cultured skin fibroblasts and amniotic fluid cells using 4-methylumbelliferyl palmitate. *Clin Chim Acta* 1978; **84**: 361.
39. Besley GTN, Broadhead DM, Lawlor E *et al*. Cholesterol ester storage disease in an adult presenting with sea-blue histiocytosis. *Clin Genet* 1984; **26**: 195.
40. Lake BD, Patrick AD. Wolman's disease: deficiency of 600-resistant acid esterase activity with storage of lipids in lysosomes. *J Pediatr* 1970; **76**: 262.
41. Kyriakides EC, Filippone N, Paul B *et al*. Lipid studies in Wolman's disease. *Pediatrics* 1970; **46**: 431.
42. Assmann G, Frederickson DS, Sloan HR *et al*. Accumulation of oxygenated sterol esters in Wolman's disease. *J Lipid Res* 1975; **16**: 28.

43. Markello TC, Guo J, Gahl WA. High-performance liquid chromatography of lipids for the identification of human metabolic disease. *Anal Biochem* 1991; **198**: 368.
44. Sloan HR, Frederickson DS. Enzyme deficiency in cholesteryl ester storage disease. *J Clin Invest* 1972; **51**: 1923.
45. Suzuki Y, Kawai S, Kobayashi A *et al*. Partial deficiency of acid lipase with storage of triglycerides and cholesterol esters in liver. *Clin Chim Acta* 1976; **69**: 219.
46. Aubert-Tulkens G, Van Hoaf F. Acid lipase deficiency: clinical and biochemical heterogeneity. *Acta Paediatr Belg* 1979; **32**: 239.
47. Young EP, Patrick AD. Deficiency of acid esterase activity in Wolman's disease. *Arch Dis Child* 1970; **45**: 664.
48. Burton BK, Emery D, Mueller HW. Lysosomal acid lipase in cultivated fibroblasts: characterization of enzyme activity in normal and enzymatically deficient cell lines. *Clin Chim Acta* 1980; **101**: 25.
49. Coates PM, Cortner JA, Hoffman GM, Brown SA. Acid lipase activity of human lymphocytes. *Biochim Biophys Acta* 1979; **572**: 225.
50. Koch GA, McAvoy M, Naylor SL *et al*. Assignment of lipase A (LIPA) to human chromosome 10. *Cytogenet Cell Genet* 1979; **25**: 176.
51. Spiegel-Adolf M, Baird HW, McCafferty M. Hematologic studies in Niemann-Pick and Wolman's disease (cytology and electrophoresis). *Confin Neurol* 1966; **28**: 399.
52. Raafat R, Hashemian MP, Abrishami MA. Wolman's disease: report of two new cases with a review of the literature. *Am J Clin Pathol* 1973; **59**: 490.
53. Uno Y, Taniguchi A, Tanaka E. Histochemical studies in Wolman's disease: report of an autopsy case accompanied with a large amount of milky ascites. *Acta Pathol Jap* 1973; **23**: 779.
54. Lajo A, Gracia R, Navarro M *et al*. Enfermedad de Wolman en su forma aguda infantil. *An Esp Pediatr* 1974; **7**: 438.
55. Lake BD. Histochemical detection of the enzyme deficiency in blood films in Wolman's disease. *J Clin Pathol* 1971; **24**: 617.
56. Kelly S, Bakhru-Kishore R. Fluorimetric assay of acid lipase in human leucocytes. *Clin Chim Acta* 1979; **97**: 239.
57. Orme RLE. Wolman's disease: an unusual presentation. *Proc R Soc Med* 1970; **63**: 489.
58. Coates PM, Cortner JA, Mennuti MT, Wheeler JE. Prenatal diagnosis of Wolman disease. *Am J Med Genet* 1978; **2**: 407.
59. Anderson RA, Sando GN. Cloning and expression of cDNA encoding human lysosomal acid lipase/cholesteryl ester hydrolase: similarities to gastric and lingual lipases. *J Biol Chem* 1991; **266**: 22 479.
60. Gasche E, Aslanidis C, Kain R *et al*. A novel variant of lysosomal acid lipase in cholesteryl ester storage disease associated with mild phenotype and improvement on lovastatin. *J Hepatol* 1997; **27**: 744.
61. Redonnet-Vernhet I, Cjatelut M, Basile JP *et al*. Cholesteryl ester storage disease: relationship between molecular defects and *in situ* activity of lysosomal acid lipase. *Biochem Mol Med* 1997; **62**: 42.
62. Anderson RA, Muruguchi Y. Mutations at the lysosomal acid lipase gene locus in patients with Wolman disease and with cholesteryl ester storage disease. *Am J Hum Genet* 1993; **53**: 882.
63. Ries S, Aslanidis C, Fehring P *et al*. A new mutation in the gene for lysosomal acid lipase leads to Wolman disease in an African kindred. *J Lipid Res* 1996; **37**: 1761.
64. Fujiyama J, Sakuraba H, Kuriyama M *et al*. A new mutation (LIPA Tyr22X) of lysosomal acid lipase gene in a Japanese patient with Wolman disease. *Hum Mutat* 1996; **8**: 377.
65. Ginsberg HN, Le N, Short MP *et al*. Suppression of apolipoprotein B production during treatment of cholesterol ester storage disease with lovastatin. *J Clin Invest* 1987; **80**: 1692.
66. Kelly DR, Hoeg JM, Demosky S, Brewer HB Jr. Characterization of plasma lipids and lipoproteins in cholesteryl ester storage disease. *Biochem Med* 1985; **33**: 29.
67. Krivit W, Freese D, Chan KW, Kulkarni R. Wolman's disease: a review of treatment with bone marrow transplantation and considerations for the future. *Bone Marrow Transplant* 1992; **10**(Suppl. 1): 97.
68. Krivit W, Peters C, Dusenbery K *et al*. Wolman disease successfully treated by bone marrow transplantation. *Bone Marrow Transplant* 2000; **26**: 567.

Fucosidosis

Introduction	740	Treatment	742
Clinical abnormalities	740	References	743
Genetics and pathogenesis	742		

MAJOR PHENOTYPIC EXPRESSION

Progressive mucopolysaccharidosis-like disease with developmental impairment, shortness of stature, coarse features, hepatosplenomegaly and dysostosis multiplex; increased sweat chloride; angiokeratomas; vacuolated lymphocytes; glycolipid storage and oligosaccharide and glycopeptide excretion; and defective activity of α -fucosidase.

INTRODUCTION

Fucose is a deoxysugar, an aldohexose in which the terminal CH₂OH is replaced by a methyl group (Figure 98.1). It occurs in glycoproteins and glycolipids as a terminal

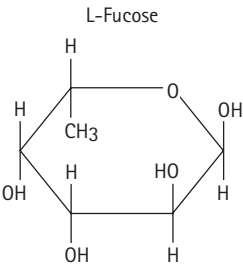


Figure 98.1 L-Fucose.

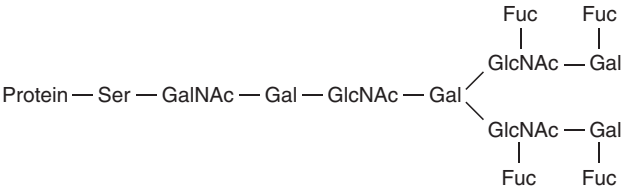


Figure 98.2 Glycoprotein structure with terminal fucose residues. The N-glycosidic linkage to amino acid could also be to threonine. In addition to the linkage shown there is N-glycosidic linkage to the asparagine residues of proteins by GalNAc. Fuc, fucose; Gal, galactose; GalNAc, N-acetylglactosamine; GlcNAc, N-acetylglucosamine; Ser, serine.

oligosaccharide linked to galactose or N-acetylglucosamine (Figure 98.2). The degradation of glycoproteins takes place sequentially in the lysosomes. Fucosidosis is a glycoprotein storage disease. Patients have impaired degradation of fucose-containing glycoproteins.

Fucosidosis was described first in 1968 by Durand and colleagues in two brothers [1, 2]. The enzyme defect was reported in the same year by Van Hoff and Hers [3]. Heterogeneity was recognized early. Most patients encountered have had the fatal infantile form of fucosidosis, but more indolent phenotypes have been reported with survival even to adulthood [4, 5]. There has been a tendency to classify these variants as type II [6] or III, with the infantile as I, but it is increasingly clear that a spectrum of mutation leads to a spectrum of variability in clinical expression [7]. The gene (FUCA1) has been mapped to chromosome 1 p34 [8, 9]. A number and variety of mutations have been identified. One mutation that causes a premature termination (Q422X) was found in eight families [10, 11], but most mutations have been unique to a single family [12, 13].

CLINICAL ABNORMALITIES

The classic infantile phenotype (Figures 98.3 and 98.4) is Hurler-like in that patients appear normal at birth but during late infancy they develop progressive coarsening of the features, and of impaired linear growth and cognitive development. Cerebral degeneration and mental deteriora-



Figure 98.3 A five-year-old Saudi girl with fucosidosis. She was mentally impaired and short and had coarse features. The cornea was hazy. Two siblings were affected.



Figure 98.4 The same patient. Her abdomen was protuberant. The tongue was large. α -Fucosidase activity of cultured fibroblasts was 0.05 percent of control.

tion progress to dementia and spasticity. There is gradual loss of muscle strength and tremor. The protuberant abdomen is a consequence of hepatosplenomegaly. The cornea may be hazy. Roentgenograms reveal the typical appearance of dysostosis multiplex (Chapter 76). Imaging of the central nervous system may reveal atrophy. The concentration of chloride in the sweat is quite high. Respiratory infection may be a problem. An end-stage decerebrate rigidity is usually followed by death within the first decade.

In the more indolent disease [7, 14] the first sign may be the development of angiokeratomas (Figure 98.5), which may be present as early as six months to four years. By 20



Figure 98.5 Angiokeratomas may be prominent, as in the inguinal area of this patient. (The illustration was kindly provided by Dr John Aase of Albuquerque, New Mexico.)

years of age they are seen in 85 percent of patients [7]. They are prominent over the buttocks and genitalia and are indistinguishable from those of Fabry disease (Chapter 88). Red streaks may be noted on the gingivae even earlier, and may be perpendicular to the roots of the teeth. There may be tortuosity of conjunctival vessels [15]. Pigmentary retinopathy has been observed [15]. The skin may appear thickened. With time, facial features become coarse, and the eyelids may be puffy. These patients may have normal sweat chloride concentrations, but they may have hypohidrosis. Hepatosplenomegaly is not characteristic. Mental deterioration is slower, and patients may live to adult life. Neurologic features include a stiff broad-based gait, spasticity, increased deep tendon reflexes and positive Babinski responses. Some patients have seizures. One patient had rapidly progressive dystonia [16]. Hearing loss has been observed [7]. Stature is reduced, but head circumference is normal [7]. The skeletal abnormalities are those of a dysostosis multiplex, which may be milder [14, 17]. The spine, pelvis and hips may be the most affected. Vertebral bodies are flattened and beaked, and there may be odontoid hypoplasia. There may be clinical kyphoscoliosis. Coxa valga is associated with flattening of the femoral heads and widened, scalloped, sclerotic acetabula. Shafts of the long bones may be wide. Neuroimaging reveals changes in the thalamus, globus pallidus, and internal capsule [18, 19].

Another phenotype [20] in which α -fucosidase was deficient was that of spondyloepiphyseal metaphyseal dysplasia. Stature was quite short, but mental development was normal. Problems in classification are highlighted by the occurrence of mild and severe presentations in the same sibship [21] and among patients homozygous for the common 422-stop mutation [7]. In addition, a patient with an initial mild appearance went on to a rapidly fatal progression [22].

Vacuolated lymphocytes are visible in the peripheral blood, and histologic examination of the liver reveals

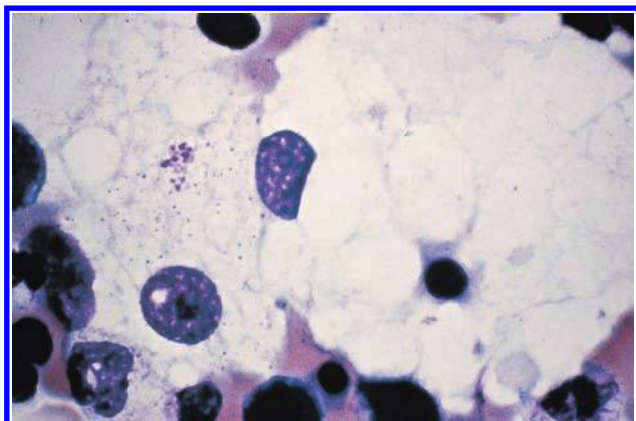


Figure 98.6 Foam cell in the marrow of a patient with fucosidosis.

foamy cytoplasm and vacuoles, some with lamellar structure [23, 24]. Foam cells are demonstrable in the bone marrow (Figure 98.6). Vacuoles may also be demonstrable in the sweat glands on skin biopsy [14], or in conjunctival cells [25]. Abnormalities have been reported in fibroblasts and in Schwann cells visualized after rectal biopsy [26]. The brain was large at autopsy and the adrenals atrophic [27]. Storage vacuoles have also been found in the ultrastructure of the brain [23].

GENETICS AND PATHOGENESIS

Fucosidosis is autosomal recessive. Consanguinity has commonly been noted. The gene for the infantile type is common in Reggio Calabria in southern Italy [28]. In the US the disease has been found in the southwest (Figure 98.5).

The gene is composed of eight exons spanning 23 kb [29]. Three patterns of mRNA were found [30] in Italian patients: two lacked mRNA; one had reduced amounts of an RNA with a cDNA by hybridization of a pattern indicating loss of a restriction site; and three had mRNA that was normal in size and content. Among mutations defined, a deletion of two exons [10] resulted in marked reduction of cross-reactive material (CRM) and absence of enzyme activity; a C-to-T transition leading to a TAA stop-codon, p.Q422X, deleting the carboxyl end of the enzyme [16, 31, 32]. This mutation causes loss of an *Eco*RI restriction site that is useful for molecular diagnosis [11]. A C to A change in exon 6 led to a stop codon p.W382X [12]. Among other mutations, most have been missense, not deletions or other major changes in gene structure [10–13, 33, 34]. Nonsense mutations were common. A patient with a missense mutation, p.L405R, was 46 years old despite less than 1 percent of control enzyme activity and no cross-reacting material [35]. Most of the mutations reported have led to virtual absence of activity of the enzyme, and this has been independent of the variability seen clinically.

Defective activity of the enzyme can be demonstrated

in leukocytes and cultured fibroblasts [14, 36–38]. Routine assays use artificial substrates and fluorimetric or colorimetric analysis. The different phenotypes cannot be distinguished by enzymatic assay as activity is essentially absent in all. There is fucosidase activity in serum or plasma, but assay is not a reliable method of diagnosis, as some normal individuals have low levels of activity in the fluid [39]. Heterogeneity among patients has been shown by the assessment of amounts of enzyme protein [40]; of 11 patients with markedly defective enzyme activity, eight made no enzyme protein in fibroblasts; in two the amounts of the 53 kDa precursor were normal, but there were no mature 50 kDa form; in one there was a small amount of CRM.

Heterozygotes tend to have activity values intermediate between patients and controls in leukocytes or fibroblasts [14, 38, 41], but there is sufficient overlap with controls that heterozygote detection and screening for carriers in a high risk population are not reliable. Prenatal diagnosis has been accomplished [41] by assay of the enzyme in cultured amniocytes. In families in which the mutation is known, this is the method of choice for prenatal diagnosis and heterozygote detection [7].

Complementation analysis of cells from patients with the different phenotypes did not yield restoration of activity [38].

A variety of fucose-containing glycolipids and glycoproteins accumulate in patients with fucosidosis. The blood groups H and Lewis are degraded with difficulty and may be present in high concentration. The H antigen glycolipid, Fuc-Gal-GlcNAc-Gal-ceramide, accumulates [42]. A variety of oligosaccharides are found in the urine [43], and this provides an approach to the initial diagnosis, although most request enzymatic analysis in a patient in whom the diagnosis is suspected. Thin layer chromatography and staining with orcinol gives a diagnostic pattern in fucosidosis, mannosidosis, sialidosis, and aspartylglycosaminuria. The glycopeptides found in the urine in fucosidosis all have GlcNAc linked to asparagine [44], often with fucose in α -1,6 linkage with GlcNAc. In addition, the fucosylGlcNAc disaccharide is found. Thin layer chromatography of the urine and staining with ninhydrin, followed by heating to 120°C, yields a bright blue spot in fucosidosis (as in aspartylglycosaminuria) that may be useful in screening [45].

TREATMENT

Only supportive treatment is available. In canine fucosidosis in Springer spaniels, bone marrow transplantation led to increased enzyme activity in neural as well as visceral tissues and reduction of storage along with clinical amelioration [46, 47]. Bone marrow transplantation in an eight-month-old patient with fucosidosis yielded a much milder degree of developmental delay 18 months later than observed in his affected sibling at the same age [48].

REFERENCES

- Durand P, Borrone C, Della Cella G, Philippart M. Fucosidosis. *Lancet* 1968; **1**: 1198.
- Durand P, Rossanna G, Borrone G. Fucosidosis. In: Durand P, O'Brien JS (eds). *Genetic Errors of Glycoprotein Metabolism*. Berlin: Springer-Verlag, 1982: 49.
- Van Hoff F, Hers HG. Mucopolysaccharidosis by absence of α -fucosidase. *Lancet* 1968; **1**: 1198.
- Patel V, Watanabe I, Zeman W. Deficiency of α -L-fucosidase. *Science* 1972; **176**: 426.
- Ikeda S, Kondo K, Oguchi K *et al*. Adult fucosidosis: histochemical and ultrastructural studies of rectal mucosa biopsy. *Neurology* 1984; **34**: 561.
- Schoondewaldt HC, Lamers KJB, Leijnen FM *et al*. Two patients with an unusual form of type II fucosidosis. *Clin Genet* 1980; **18**: 348.
- Willems PJ, Gatti R, Darby JK *et al*. Fucosidosis revisited: A review of 77 patients. *Am J Med Genet* 1991; **38**: 111.
- Carritt B, King J, Welch HM. Gene order and localization of enzyme loci on the short arm of chromosome 1. *Ann Hum Genet* 1982; **46**: 329.
- Fowler ML, Nakai H, Byers MG *et al*. Chromosome 1 localization of the human alpha-L-fucosidase structural gene with a homologous site on chromosome 2. *Cytogenet Cell Genet* 1986; **43**: 103.
- Willems PJ, Darby JK, DiCioccio RA *et al*. Identification of a mutation in the structural α -L-fucosidase gene in fucosidosis. *Am J Hum Genet* 1988; **43**: 756.
- Kretz KA, Darby JK, Willems PJ, O'Brien JS. Characterization of EcoRI mutation in fucosidosis patients: A stop codon in the open reading frame. *J Mol Neurosci* 1989; **1**: 177.
- Seo H-C, Willens PJ, Kretz KA *et al*. Fucosidosis: Four new mutations and a new polymorphism. *Hum Mol Genet* 1993; **2**: 423.
- Seo HC, Meiheng Y, Kim AH *et al*. A 66-basepair insertion in exon 6 of the α -L-fucosidase gene of a fucosidosis patient. *Hum Mutat* 1996; **7**: 183.
- Kousseff BG, Beratis NG, Strauss L *et al*. Fucosidosis Type 2. *Pediatrics* 1976; **57**: 205.
- Snodgrass MB. Ocular findings in a case of fucosidosis. *Br J Ophthalmol* 1976; **60**: 508.
- Gordon BA, Gordon KE, Seo HC *et al*. Fucosidosis with dystonia. *Neuropediatrics* 1995; **26**: 325.
- Brill PW, Beratis NG, Kousseff BG, Hirschhorn K. Roentgenographic findings in fucosidosis type 2. *Am J Roentgen* 1975; **124**: 75.
- Provenzale JM, Barboriak DP, Sims K. Neuroradiologic findings in fucosidosis a rare lysosomal storage disease. *Am J Neuroradiol* 1995; **16**: 809.
- Terespolsky D, Clarke JTR, Blaser SI. Evolution of the neuroimaging changes in fucosidosis type II. *J Inherit Metab Dis* 1996; **19**: 775.
- Schafer IA, Powell DW, Sullivan JC. Lysosomal bone disease. *Pediatr Res* 1971; **5**: 391.
- Fleming C, Rennie A, Fallowfield M, McHenry PM. Cutaneous manifestations of fucosidosis. *Br J Dermatol* 1997; **136**: 594.
- Bock A, Fang-Kircher S, Braun F *et al*. Another unusual case of fucosidosis. *J Inherit Metab Dis* 1995; **18**: 93.
- Loeb H, Tondeur M, Jonniaux G *et al*. Biochemical and ultrastructural studies in a case of mucopolysaccharidosis 'F' (fucosidosis). *Helv Paediatr Acta* 1969; **24**: 519.
- Freitag F, Kuchemann K, Blumcke S. Hepatic ultrastructure in fucosidosis. *Virchows Arch (B)* 1971; **7**: 99.
- Libert J, Van Hoof F, Tondeur M. Fucosidosis: Ultrastructural study of conjunctiva and skin and enzyme analysis of tears. *Invest Ophthalmol* 1976; **15**: 626.
- Ikeda S, Kondo K, Oguchi K *et al*. Adult fucosidosis: histochemical and ultrastructural studies of rectal mucosa biopsy. *Neurology* 1984; **34**: 451.
- Larbrisseau A, Bouchu P, Jasmin G. Fucosidose de type I: Etude anatomique. *Arch Fr Pediatr* 1979; **36**: 1013.
- Sangiorgi S, Mochi M, Beretta M *et al*. Genetic and demographic characterization of a population with high incidence of fucosidosis. *Hum Hered* 1982; **32**: 100.
- Kretz KA, Cripe D, Carson GS *et al*. Structure and sequence of the human α -L-fucosidase gene and pseudogene. *Genomics* 1992; **12**: 276.
- Guazzi S, Persici P, Gatti R *et al*. Heterogeneity of mRNA expression in Italian fucosidosis patients. *Hum Genet* 1989; **82**: 63.
- Kretz KA, Darby JK, Willems PJ *et al*. Characterization of EcoRI mutation in fucosidosis patients: A stop codon in the open reading frame. *J Mol Neurosci* 1989; **1**: 177.
- Yang M, Allen H, DiCioccio RA. Pedigree analysis of α -L-fucosidase gene mutations in a fucosidosis family. *Biochem Biophys Acta* 1993; **1182**: 245.
- Cragg H, Williamson M, Young E *et al*. Fucosidosis: Genetic and biochemical analysis of eight cases. *J Med Genet* 1997; **34**: 105.
- Seo HC, Yang M, Tonlorenzi R *et al*. A missense mutation (S63L) in α -L-fucosidase is responsible for fucosidosis in an Italian patient. *Hum Mol Genet* 1994; **3**: 2065.
- Fleming CJ, Sinclair DU, White EJ *et al*. A fucosidosis patient with relative longevity and a missense mutation in exon 7 of the alpha-fucosidase gene. *J Inherit Metab Dis* 1998; **21**: 688.
- Zielke K, Veath ML, O'Brien JS. Fucosidosis: Deficiency of α -L-fucosidase in cultured skin fibroblasts. *J Exp Med* 1972; **136**: 197.
- Wood S. A sensitive fluorometric assay for α -L-fucosidase. *Clin Chem Acta* 1975; **58**: 251.
- Beratis NG, Turner BM, Labadie G, Hirschhorn K. α -L-Fucosidase in cultured skin fibroblasts from normal subjects and fucosidosis patients. *Pediatr Res* 1977; **11**: 862.
- Wood S. Human α -L-fucosidase: A common polymorphic variant for low serum enzyme activity studies of serum and leukocyte enzyme. *Hum Hered* 1979; **29**: 226.
- Johnson K, Dawson G. Molecular defect in processing α -fucosidase in fucosidosis. *Biochem Biophys Res Commun* 1985; **133**: 90.

41. Durand P, Gatti R, Borrone C *et al.* Detection of carriers and prenatal diagnosis for fucosidosis in Calabria. *Hum Genet* 1979; **51**: 195.
42. Dawson G, Spranger JW. Fucosidosis: A glycosphingolipidosis. *N Engl J Med* 1971; **285**: 122.
43. Holmes EW, O'Brien JS. Separation of glycoprotein-derived oligosaccharides by thin-layer chromatography. *Anal Biochem* 1979; **93**: 167.
44. Yamashita K, Tachibana Y, Takada S *et al.* Urinary glycopeptides of fucosidosis. *J Biol Chem* 1979; **254**: 4820.
45. Simell O, Sipila I, Autio S. Extra heating of TLC plates detects two lysosomal storage diseases aspartylglucosaminuria and fucosidosis during routine urinary amino acid screening. *Clin Chem Acta* 1983; **133**: 227.
46. Taylor RM, Farrow BRH, Stewart GH, Healy PJ. Enzyme replacement in nervous tissue after allogeneic bone-marrow transplantation for fucosidosis in dogs. *Lancet* 1986; **II**: 772.
47. Taylor RM, Farrow BRH, Stewart GJ. Amelioration of clinical disease following bone marrow transplantation in fucosidase-deficient dogs. *Am J Med Genet* 1992; **42**: 628.
48. Vellodi A, Cragg H, Winchester B *et al.* Allogeneic bone marrow transplantation for fucosidosis. *Bone Marrow Transplant* 1995; **15**: 153.

α-Mannosidosis

Introduction	745	Treatment	748
Clinical abnormalities	745	References	749
Genetics and pathogenesis	748		

MAJOR PHENOTYPIC EXPRESSION

Severe mental and motor impairment with deterioration and early death; coarse features; hepatosplenomegaly; dysostosis multiplex; cataracts and corneal opacities; deafness, storage of mannosylglycoproteins, urinary excretion of mannosyl-oligosaccharides and defective activity of α-mannosidase.

INTRODUCTION

Patients have been increasingly recognized in which the clinical features were those of mucopolysaccharidosis, but there was no mucopolysacchariduria. The recognition of inclusions set out I-cell disease (Chapter 83) as a distinct entity in 1967. In the same year, Öckerman [1] described α-mannosidosis. The enzyme (Figure 99.1) exists in at least two forms, which are immunologically indistinguishable and are coded for by a single gene, MAN2B1, on chromosome 19 p13–q12 [2]. The gene has been sequenced [3–5]. A mutation, 212A>T, was found in two siblings of a consanguineous mating [6]. Other

mutations in a full spectrum of mutation types have been reported [7, 8].

CLINICAL ABNORMALITIES

Clinical features in more than 90 patients with the disease have reflected considerable phenotypic diversity [9–17]. Patients have been classified into the severe infantile type of disease and a more indolent form. It is already clear that there is a spectrum including a wide variety of expression. Phenotypic heterogeneity within sibships has also been described [18], which means that there are modifiers of expression and that certainly classification into two forms is simplistic.

The infantile or classic form of mannosidosis is characterized by a very early onset of a disease that resembles a severe mucopolysaccharidosis (Figures 99.2, 99.3, 99.4, and 99.5). Hernias may be among the earliest findings. Facial features are very coarse. The skin may feel thickened, indicating the presence of stored material. Hepatosplenomegaly is prominent. There may be noisy breathing, nasal discharge or frequent respiratory infections. Macrocephaly is present, along with frontal bossing [13]. Mental development is severely impaired. Speech development may be worse – a consequence of impaired hearing. Gait may be broad-based.

Dysostosis multiplex is extreme [19]. The sella turcica is J-shaped and the calvaria thickened. Vertebral bodies are hypoplastic and flattened or ovoid with anterior

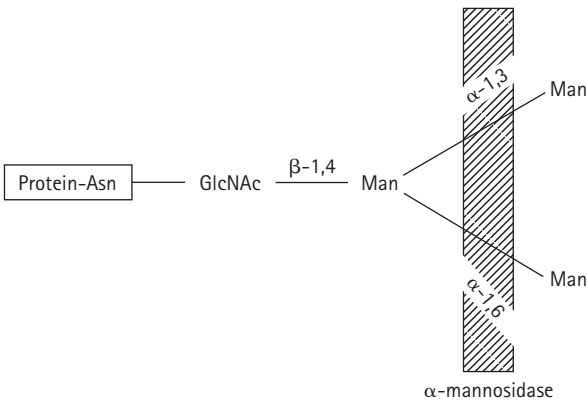


Figure 99.1 The mannosidase reaction. Asn, asparagine; GlcNAc, N-acetylglucosamine; Man, mannose.



Figure 99.2 AMR: A 20-month-old Saudi Arabian boy with mannosidosis. At this age, he could stand and cruise, but did not walk or speak. The activity of α -mannosidase in fibroblasts was 10 percent of control.



Figure 99.3 AMR: The facies of this patient, especially the lips and nose, suggested the presence of storage material.

beaking. A gibbus may be present. Proximal metacarpals are tapered and the iliac wings flared. Pulmonary infiltrates are commonly seen. There may be corneal opacities and posterior lenticular cataracts in a spoke-like pattern



Figure 99.4 AMR: The abdomen was protuberant. The hepatomegaly is outlined. The spleen was also enlarged. Bone marrow revealed foamy histiocytes.



Figure 99.5 AMR: He had clinodactyly of the fifth fingers and proximally placed thumbs.

[20–22]. Deterioration may be rapid, and most patients die between three and ten years of age, often of pneumonia.

Other patients may have a more indolent course. Major features are impaired mental development and hearing loss [23–26] (Figures 99.6 and 99.7). Survival into adulthood is common. Some of these patients have mild dysostosis multiplex, while others do not [24]. Other skeletal problems include kyphoscoliosis (Figure 99.6) and destructive synovitis of the knees [26, 27]. Hepatosplenomegaly may be absent. The eyes are usually clear, but retinal degeneration has been reported [7]. Hearing loss is progressive, and storage material has been found in the ear (Figure 99.7). Hydrocephalus has been reported [28] and spastic paraplegia [29], as well as ataxia [7]. Progressive cerebellar ataxia of late adolescent onset was the initial clinical manifestation in three adult siblings [30]. They also displayed nystagmus, dysarthria, and positive Babinski responses. Hyperphagia has been observed [17]. Magnetic resonance imaging (MRI) findings have included cerebellar atrophy and abnormal signal in



Figure 99.6 Roentgenogram of the spine of a patient with mannosidosis shows scoliosis. There was generalized dysostosis multiplex. (Illustration kindly provided by Dr Philip Benson.)



Figure 99.7 DF: A 15-year-old with a mild variant of mannosidosis. She had a learning disability from infancy and had an IQ of 78. She had bilateral hearing loss. Surgery on the right middle ear at 19 years revealed extensive deposits of mannoside.

the white matter [31]. Cardiovascular abnormality has been observed in premature ventricular contractions and a shortened PR interval on electrocardiogram (ECG) [32].

Hematological evaluation regularly reveals vacuolated

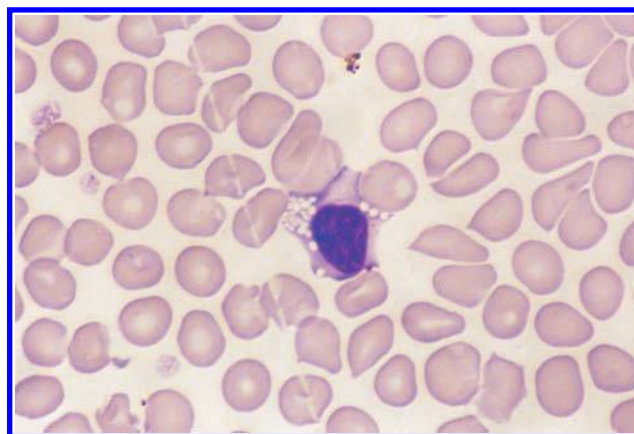


Figure 99.8 DF: Vacuolated lymphocytes from peripheral blood.

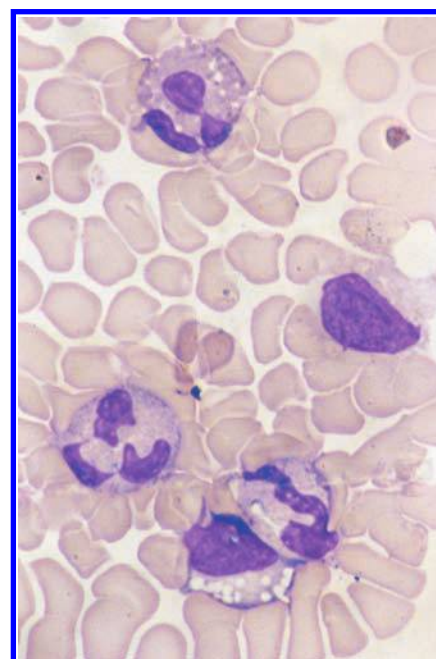


Figure 99.9 DF: Polymorphonuclear leukocytes were also vacuolated.

lymphocytes in patients with all types of mannosidosis [13, 24] (Figure 99.8). Polymorphonuclear leukocytes may also be vacuolated (Figure 99.9), and the bone marrow may reveal foamy macrophages. Pancytopenia has been reported [33]. Increased susceptibility to infections, both bacterial and viral including reported bronchitis and otitis media, has been documented and associated with a variety of abnormalities in leukocyte function [13, 34], including defective response to chemotactic stimulation and slowed phagocytosis. The ability of lymphocytes to undergo transformation was reduced. There may be some reduction in IgG.

Histopathology has revealed foamy, vacuolated hepatocytes [9, 13, 28, 29]. Vacuoles have also been observed in

histiocytes, lymphocytes [16], muscle, and fibroblasts [7]. Neuronal changes were widespread in the central nervous system [13, 35], with ballooning and ultrastructural evidence of storage vacuoles.

GENETICS AND PATHOGENESIS

α -Mannosidosis is an autosomal recessive disease, and affected offspring have been of both sexes [8]. The gene has been assigned to chromosome 9 [36] to the central region between p13.2 and q12 [2, 37]. It contains 24 exons. The mutation at nucleotide 212 leads to the H71L amino acid change [6]. Two other mutations were reported [38] in two homozygous Italian patients with α -mannosidosis, IVS-2A>G and 322-323insA. The first led to skipping of exon 21. The second caused a frame shift with a stop codon at amino acid 160. A 47-year-old Japanese woman with a homozygous C>T change in exon 19 leading to R760X had less than 1 percent of 99 control enzyme activities [7]. Q639X and R750W were found in a seven-year-old Finnish boy [7].

The defective enzyme, acid α -mannosidase (Figure 99.1), is lysosomal and is synthesized in a precursor form followed by processing into smaller subunits assembled in human liver into forms, which are separable by chromatography and electrophoresis but are immunologically indistinguishable [8, 13, 39–41]. Residual activity in affected patients usually ranges between 3 and 5 percent of control [15, 25, 42]. Some variant patients have had higher (15–20 percent) residual activity [25, 42]. Levels in leukocytes tend to be lower than those in fibroblasts, but the diagnosis can be made with either. The diagnosis has also been evident on assay of the enzyme in plasma, but this is not recommended as reliable [13]. Immunologic cross-reactive material appears to be present in most patients [15, 42].

Prenatal diagnosis has been carried out by assay of the enzyme in cultured amniocytes or in chorionic villus material [43–49]. Normal activity in chorionic villi may be considerably less than in amniocytes [47]. Accurate prenatal diagnosis must take into account not only the issue of variant residual activity, but forms of α -mannosidase that are not defective in patients with mannosidosis [41].

Heterozygotes may have intermediate levels of enzyme activity, but they are more often normal [13, 15]; therefore, this is not reliable. If the mutation is known, molecular analysis is the method of choice for both prenatal and heterozygote detection.

The result of the defective enzymatic activity is the storage of a variety of glycoproteins and glycoprotein-derived oligosaccharides. These have been best characterized in the urine [48–50], and it is by study of the patterns of urinary oligosaccharides (Figures 99.10 and 99.11) that the diagnosis has usually been first made chemically, mainly by thin layer chromatography

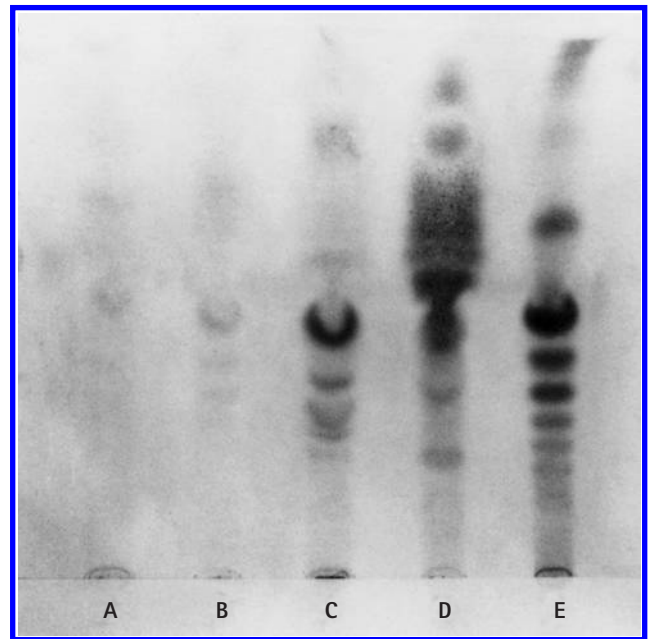


Figure 99.10 Thin layer chromatogram of urinary oligosaccharides. Lane A was normal urine; lane B the patient, and lane C a patient with classic mannosidosis. Lanes D and E were the normal and patient 20x concentrated, indicating the presence in DF of smaller concentrations of the oligosaccharides than seen in unconcentrated urine in the more classic mannosidosis phenotype. (This illustration and Figure 99.11 were kindly provided by Dr Thomas G Warner and are printed with permission from *Clin Genet* 1984; 25: 248.)

(Figure 99.10) [15, 24, 51, 52]. There are a number of mannosyloligosaccharides in the urine of these patients. The major one is the trisaccharide, Man- α (1,3)Man- β (1,4)GlcNAc [24, 53, 54] (Figure 99.11).

Affected patients, and those with aspartylglucosaminuria, have been reported to have elevated levels of dolichol in the serum [55, 56]. This could prove useful in diagnosis. It may reflect the fact that complex glycoproteins are synthesized by the transfer of oligosaccharide precursor from dolichol to the asparagine of the peptide.

TREATMENT

Effective specific treatment for mannosidosis has not been reported. Bone marrow transplantation has potential utility [57].

β -Mannosidosis

Another disorder of glycoprotein catabolism, β -mannosidosis, was first described in goats who displayed a severe degree of neurodegeneration [58]. They had defective activity of β -mannosidase.

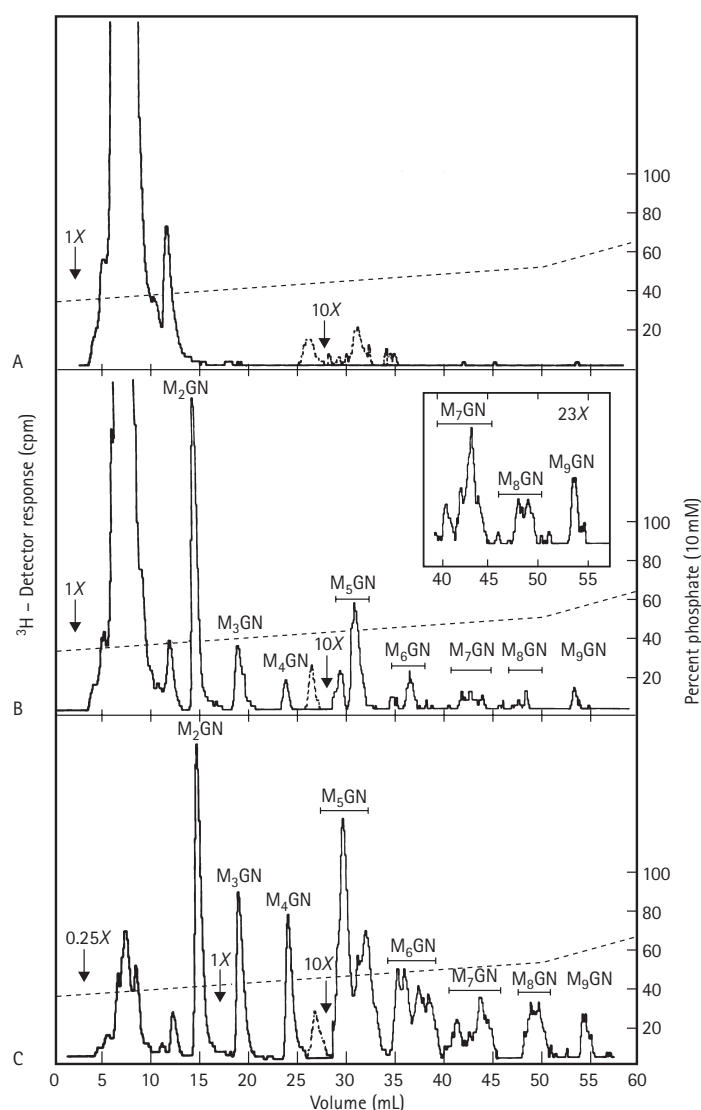


Figure 99.11 HPLC chromatogram of urinary oligosaccharides as 3H-aldols. A was normal urine, B the patient and C half as much urine of the classic patient. The mannose containing fractions were labeled M, e.g. M₂GN, the major fraction, was the trisaccharide Man- α (1-3)Man- β (1-4)GlcNAc.

It is now apparent that this disease also occurs in man. Severity of disease is variable but may be mild [59]. An example was a 44-year-old man with impaired mental development and no other neurologic abnormalities, but he had scrotal angiokeratomas [60]. Deafness and speech impairment were reported in two consanguineous Turkish siblings [61]. An 18-year-old patient with bilateral hearing loss and mild cognitive impairment also had symptoms of Tourette syndrome [62].

Mutations in the MANBA gene have been found in many of these families, many of them null mutations [59, 62, 63].

REFERENCES

- Öckerman P-A. A generalized storage disorder resembling Hurler's syndrome. *Lancet* 1967; **2**: 239.
- Kaneda Y, Hayes H, Uchida T *et al*. Regional assignment of five genes on human chromosome 19. *Chromosoma* 1987; **95**: 8.
- Liao YF, Lal A, Moreman KW. Cloning expression purification and characterization of the human broad specificity lysosomal acid α -mannosidase. *J Biol Chem* 1996; **271**: 28 348.
- Wakamatsu N, Gotoda Y, Saito S, Kawai H. Characterization of the human MANB gene encoding lysosomal α -D-mannosidase. *Gene* 1997; **198**: 351.
- Riise HMF, Berg T, Nilssen O *et al*. Genomic structure of the human lysosomal α -mannosidase gene (MANB). *Genomics* 1997; **42**: 200.
- Nilssen O, Berg T, Riise HMF *et al*. α -Mannosidosis: functional cloning of the lysosomal α -mannosidase cDNA and identification of a mutation in two affected siblings. *Hum Mol Genet* 1997; **6**: 717.
- Gotoda Y, Wakamatsu N, Kawai H. Missense and nonsense mutations in the lysosomal alpha-mannosidase gene (MANB) in severe and mild forms of alpha-mannosidosis. *Am J Hum Genet* 1998; **63**: 1015.
- Berg T, Riise HMF, Hansen GM *et al*. Spectrum of mutations in alpha-mannosidosis. *Am J Hum Genet* 1999; **64**: 77.
- Kjellman B, Gamstorp I, Brun A *et al*. Mannosidosis: a clinical and histopathologic study. *J Pediatr* 1969; **75**: 366.
- Tsay GC, Dawson G, Matalon R. Excretion of mannose-rich complex carbohydrates by a patient with α -mannosidase deficiency (mannosidosis). *J Pediatr* 1974; **84**: 865.
- Farriaux JP, Legouis I, Humbel R *et al*. La mannosidose: A propos de 5 observations. *Nouv Presse Med* 1975; **4**: 1867.
- Aylsworth AS, Taylor HA, Stuart CF, Thomas GH. Mannosidosis: phenotype of severely affected child and characterization of α -mannosidase activity in cultured fibroblasts from the patient and his parents. *J Pediatr* 1976; **88**: 814.
- Desnick RJ, Sharp HL, Grabowski GA *et al*. Mannosidosis: clinical morphologic immunologic and biochemical studies. *Pediatr Res* 1976; **19**: 985.
- Yunis JJ, Lewandowski RC, Sanfilippo SJ *et al*. Clinical manifestations of mannosidosis – a longitudinal study. *Am J Med* 1976; **61**: 841.
- Warner TG, O'Brien JS. Genetic defects in glycoprotein metabolism. *Ann Rev Genet* 1990; **17**: 395.
- Bennett JK, Dembure PP, Elsas LJ. Clinical and biochemical analysis of two families with type I and type II mannosidosis. *Am J Med Genet* 1995; **55**: 21.
- Owayed A, Clark JTR. Hyperphagia in patients with α -mannosidosis type II. *J Inher Metab Dis* 1997; **20**: 727.
- Mitchell ML, Erickson RP, Schmid D *et al*. Mannosidosis: two brothers with different degrees of disease severity. *Clin Genet* 1981; **20**: 191.
- Spranger J, Gehler J, Cantz M. The radiographic features of mannosidosis. *Radiology* 1976; **119**: 401.
- Murphree AL, Beaudet AL, Palmer EA, Nichols BL. Cataract in mannosidosis. *Birth Defects* 1976; **12**: 319.

21. Arbisser AL, Murphree AL, Garcia CA, Howell RR. Ocular findings in mannosidosis. *Am J Ophthalmol* 1976; **82**: 465.
22. Letson RD, Desnick RJ. Punctate lenticular opacities in type II mannosidosis. *Am J Ophthalmol* 1978; **85**: 218.
23. Booth CW, Chen KK, Nadler HL. Mannosidosis: clinical biochemical and ultrastructural studies in a family of affected adolescents and adults. *J Pediatr* 1976; **88**: 821.
24. Warner TG, Mock AK, Nyhan WL, O'Brien JS. α -Mannosidosis: analysis of urinary oligosaccharides with high performance liquid chromatography and diagnosis of a case with unusually mild presentation. *Clin Genet* 1984; **25**: 248.
25. Bach G, Kohn G, Lasch EE *et al.* A new variant of mannosidosis with increased residual enzymatic activity and mild clinical manifestation. *Pediatr Res* 1978; **12**: 1010.
26. Montgomery TR, Thomas GH, Valle DL. Mannosidosis in an adult. *Johns Hopkins Med J* 1982; **151**: 113.
27. Weiss SW, Kelly WD. Bilateral destructive synovitis associated with alpha mannosidase deficiency. *Am J Surg Pathol* 1983; **7**: 487.
28. Halperin JL, Landis DMD, Weinstein LA *et al.* Communicating hydrocephalus and lysosomal inclusions in mannosidosis. *Arch Neurol* 1984; **41**: 777.
29. Kawai H, Nishino H, Nishida Y *et al.* Skeletal muscle pathology of mannosidosis in two siblings with spastic paraplegia. *Acta Neuropathol (Berl)* 1985; **68**: 201.
30. Gutschalk A, Harting I, Cantz M *et al.* Adult alpha-mannosidosis: clinical progression in the absence of demyelination. *Neurology* 2004; **63**: 1744.
31. Dietemann L, Filippi de la Palavesa MM, Tranchant C, Kastler B. MR findings in mannosidosis. *Neuropathology* 1990; **32**: 485.
32. Mehta J, Desnick RJ. Abbreviated PR interval in mannosidosis. *J Pediatr* 1978; **92**: 599.
33. Press OW, Fingert H, Lott IT, Dickersin CR. Pancytopenia in mannosidosis. *Arch Intern Med* 1983; **143**: 1268.
34. Quie PG, Cates KL. Clinical conditions associated with defective polymorphonuclear leukocyte chemotaxis. *Am J Pathol* 1977; **88**: 711.
35. Sung JH, Hayano M, Desnick RJ. Mannosidosis: pathology of the nervous system. *J Neuropathol Exp Neurol* 1977; **36**: 807.
36. Champion MJ, Shows TB. Mannosidosis: assignment of the lysosomal α -mannosidase B gene to chromosome 19 in man. *Proc Natl Acad Sci USA* 1977; **74**: 455.
37. Martinvik F, Ellenbogen A, Hirschhorn K, Hirschhorn R. Further localization of the gene for human acid alpha glucosidase (GAA) peptidase D (PEPD) and α -mannosidase B (MANB) by somatic cell hybridization. *Hum Genet* 1985; **69**: 109.
38. Beccari T, Bibi L, Ricci R *et al.* Two novel mutations in the gene for human α -mannosidase that cause α -mannosidosis. *J Inherit Metab Dis* 2003; **26**: 819.
39. Carroll M, Dance N, Masson PK *et al.* Human mannosidosis – the enzyme defect. *Biochem Biophys Res Commun* 1972; **49**: 579.
40. Pohlmann R, Hasilik A, Cheng S *et al.* Synthesis of lysosomal α -mannosidase in normal and mannosidosis fibroblasts. *Biochem Biophys Res Commun* 1983; **115**: 1083.
41. Cheng SH, Malcolm S, Pemble S, Winchester B. Purification and comparison of the structures of human liver acidic α -D-mannosidase A and B. *Biochem J* 1986; **233**: 65.
42. Poenaru L, Miranda C, Dreyfus J-C. Residual mannosidase activity in human mannosidosis. Characterization of the mutant enzyme. *Am J Hum Genet* 1980; **32**: 354.
43. Maire I, Zabet MT, Mathieu M, Cotte J. Mannosidosis: tissue culture studies in relation to prenatal diagnosis. *J Inherit Metab Dis* 1978; **1**: 19.
44. Poenaru L, Girard S, Thepot F *et al.* Antenatal diagnosis in three pregnancies at risk for mannosidosis. *Clin Genet* 1979; **16**: 428.
45. Poenaru L, Kaplan L, Dummies J, Dreyfus JC. Evaluation of possible first trimester prenatal diagnosis in lysosomal diseases by trophoblast biopsy. *Pediatr Res* 1984; **18**: 1032.
46. Petushkova NA. First-trimester diagnosis of an unusual case of α -mannosidosis. *Prenat Diagn* 1991; **11**: 279.
47. Fukuda M, Tanaka A, Ishiki G. Variation of lysosomal enzyme activity with gestational age in chorionic villi. *J Inherit Metab Dis* 1990; **13**: 862.
48. Yamashita K, Tachibana Y, Mihara K *et al.* Urinary oligosaccharides of mannosidosis. *J Biol Chem* 1979; **255**: 5126.
49. Matsuura F, Nunez HA, Grabowski GA, Sweeley CC. Structural studies of urinary oligosaccharides from patients with mannosidosis. *Arch Biochem Biophys* 1981; **207**: 337.
50. Kistler JP, Lott IT, Kolodny EH *et al.* Mannosidosis: new clinical presentation enzyme studies and carbohydrate analysis. *Arch Neurol* 1977; **34**: 45.
51. Humbel R, Collart M. Oligosaccharides in urine of patients with glycoprotein storage diseases. *Clin Chim Acta* 1975; **60**: 143.
52. Sewell AC. An improved thin layer chromatographic method for urinary oligosaccharide screening. *Clin Chim Acta* 1979; **92**: 411.
53. Nordén NE, Lundblad A, Svenson S, Autio S. Characterization of two mannose-containing oligosaccharides isolated from the urine of patients with mannosidosis. *Biochemistry* 1974; **13**: 871.
54. Nordén NE, Lundblad A. A mannose-containing trisaccharide isolated from urines of three patients with mannosidosis. *J Biol Chem* 1973; **248**: 6210.
55. Salaspuro M, Salmela K, Humaloja K *et al.* Elevated level of serum dolichol in aspartylglucosaminuria. *Life Sci* 1990; **47**: 627.
56. Humaloja K, Roine RP, Salmela K *et al.* Serum dolichols in different clinical conditions. *Scand J Clin Lab Invest* 1991; **51**: 705.
57. Walkley SU, Thrall MA, Dobrenis K *et al.* Bone marrow transplantation corrects the enzyme defect in neurons of the central nervous system in a lysosomal storage disease. *Proc Natl Acad Sci USA* 1994; **91**: 2970.
58. Jones MZ, Dawson G. Caprine beta-mannosidosis: inherited deficiency of beta-D-mannosidase. *J Biol Chem* 1981; **256**: 5185.
59. Bedilu R, Nummy KA, Cooper A. Variable clinical presentation of lysosomal beta-mannosidosis in patients with null mutations. *Molec Genet Metab* 2002; **77**: 282.
60. Cooper A, Sardharwalla IB, Roberts MM. Human beta-mannosidase deficiency. *N Engl J Med* 1986; **315**: 1231.
61. Dorland L, Duran M, Hoefnagels FET *et al.* Beta-mannosidosis in two brothers with hearing loss. *J Inherit Metab Dis* 1988; **11**: 255.

62. Sedel F, Friderici K, Nummy K. Atypical Gilles de la Tourette syndrome with beta-mannosidase deficiency. *Arch Neurol* 2006; **63**; 129.
63. Rilse HMF, Persichetti E, Sorriso C. Identification of two novel beta-mannosidosis-associated sequence variants: biochemical analysis of beta-mannosidase (MANBA) missense mutations. *Molec Genet Metab* 2008; **94**; 476.

Galactosialidosis

Introduction	752	Treatment	756
Clinical abnormalities	753	References	757
Genetics and pathogenesis	755		

MAJOR PHENOTYPIC EXPRESSION

Early infantile

Edema, fetal (hydrops fetalis)-neonatal; telangiectases; hepatosplenomegaly; growth failure; psychomotor delay and deterioration; dystosis multiplex; cardiac failure; proteinuria; and death in infancy.

Late infantile

Hepatosplenomegaly; dystosis multiplex; corneal clouding; hernias; valvular cardiac disease; shortness of stature and hearing loss.

Juvenile (adult)

Ataxia; myoclonus; seizures; impaired mental development and deterioration; cherry red spots; corneal clouding; angiokeratomas; platyspondyly; and shortness of stature.

Each type

Coarse facies; cherry red spots; foam cells; defective activity of β -galactosidase and neuraminidase resulting from abnormality in the lysosomal protective protein/cathepsin A (PPCA).

INTRODUCTION

Following the discovery in 1968 of β -galactosidase deficiency in generalized GM₁ gangliosidosis [1], a number of patients were reported with atypical features such as cherry red macular spots and an absence of hepatosplenomegaly [2–5]. The combination of features of cerebral lipidosis and mucopolysaccharidosis without mucopolysacchariduria suggested a mucolipidosis. Complementation studies in somatic cell hybrids indicated that two of these patients [4, 5] had mutations distinct from or non-allelic with GM₁ gangliosidosis, though they were clearly deficient in β -galactosidase [6]. Wenger *et al.* [7] in 1978 reported the coexisting deficiency of neuraminidase and β -galactosidase in leukocytes and fibroblasts of a patient with what had been thought [8] to be a variant form of GM₁ gangliosidosis. Then other patients were reported in whom sialidase deficiency was present [9–12] along with that of β -galactosidase.

That the primary defect was not in sialidase was shown

by complementation of the cells of patients with sialidosis by hybridization with cells of patients with the combined defect [11, 13]. Further, in the combined defect cells, both enzyme defects could be restored by a glycoprotein corrective factor produced in culture by normal fibroblasts or those of β -galactosidase deficiency indicating the presence of a third protein acting as a corrective factor. The turnover of β -galactosidase in normal fibroblasts was 10 days, while in the cells deficient in both enzymes it was less than 1 day [14], and in experiments with purified enzyme it was clear that the rapid turnover was caused by proteolytic degradation of the enzyme [15, 16]. The disorder was named galactosialidosis in 1981 [17]. The molecular defect was found by D'Azzo and colleagues [16] to be in the PPCA, which aggregates with both enzymes to form multimers that resist lysosomal degradation. The gene has been mapped to chromosome 20q13.1 [18, 19], and the human cDNA has been cloned [20]. Mutations have been discovered [21, 22].

Multienzyme complexes may work synergistically and

provide more efficient responses to changes in the load and composition of substrates. The glycoprotein storage diseases, β -galactosidase deficiency, neuraminidase deficiency, and PPCA, the defect in galactosialidosis, are representative of such a multiprotein complex [23]. In contrast, GM1-gangliosidosis is a glycosphingolipids storage disease. Both are components of the lysosomal network of organelles involved in sorting, digestion, recycling, and secretion of cellular components. The functional mutations in each component of the glycoprotein complex result in impressive lysosomal storage disorders. The multiprotein complex includes the protective cathepsin A protein in which mutations lead to galactosialidosis. The protective protein protects both enzymes from proteolytic degradation. Mutations have been reported in the PPCA gene [24]. Features of GM₁ gangliosidosis and sialidosis result from defective activity of both β -galactosidase and neuraminidase as a result of fundamental deficiency in the lysosomal PPCA, which is itself a serine carboxypeptidase.

CLINICAL ABNORMALITIES

Considerable phenotypic heterogeneity has been observed consistent with different mutations. Nevertheless, it continues to appear useful to distinguish the early infantile and late infantile phenotypes, while the rest, accounting for 70 percent of the patients, have been called the juvenile/adult type and appear to represent quite a broad spectrum of variants.



Figure 100.1 A newborn infant with galactosialidosis who presented with non-immune hydrops fetalis and had extreme edema of the vulva.



Figure 100.2 Close-up of the edematous genitalia. A mutation V132M was found by analysis of the DNA by Dr Suzuki.



Figure 100.3 Another infant with hydrops fetalis.

The early infantile is the most severe form of the disease. Fetal hydrops may lead to stillbirth or early neonatal death [24]; extensive edema may be evident in the neonatal period (Figures 100.1, 100.2, and 100.3). Features are coarse [25–30] (Figures 100.4 and 100.5), and there is hepatosplenomegaly. Inguinal hernias are common. Psychomotor delay may be global, and deterioration is progressive to death at an average age of seven months.



Figure 100.4 A seven-month-old Omani child with galactosialidosis. The features were quite coarse and the eyebrows abundant. There was a substantial amount of hair on the head despite almost complete absence of subcutaneous tissue. There were cherry red macular spots



Figure 100.5 A one-year-old with galactosialidosis. Impaired development was global. The liver and spleen were enlarged and there were cherry red macular spots.

Dysostosis multiplex is uniformly present; it may be less prominent than in other forms of the dysostosis because of the short interval in which to develop before demise. Telangiectases have been found in the early infantile disease, but angiokeratomas are rarely seen. There may be corneal clouding and cherry red spots [25]. Proteinuria is an early sign of renal dysfunction; renal failure may ensue [29]. Infiltration of the heart leads to thickened septa, cardiomegaly, and congestive failure may occur as early as the first week of life [25–27, 31]. Recurrent fetal hydrops has been reported in two families [32, 33]. Thrombocytopenia with purpura and anemia were reported in a patient with fetal hydrops [34]. Anemia and thrombocytopenia were also found, along with hemophagocytosis in a seven-month-old boy [35].

The late infantile form of the disease may be evident as early as the first month of life. Patients have coarse features, hepatosplenomegaly, and dysostosis multiplex with the appearance of mucopolysaccharidosis [5, 17, 36–38]. Some have had cherry red spots and/or corneal clouding [17, 37]; others have not [5, 36]. Generalized seizures or petit mal has rarely been observed [5]. Impaired mental development in these patients has generally been mild. Neurologic deterioration has not generally been seen [5]. Cardiac involvement is a regular feature of the disease. Valvular involvement has included thickened mitral and aortic valves. Hearing loss may be conductive or mixed. Shortness of stature may be a consequence of disease of the spine, and there may be atrophy of the muscles. Angiokeratomas are uncommon.



Figure 100.6 A 5-year-old boy with galactosialidosis. He had mildly impaired mental development and had mildly coarse features and hirsutism. Dr Suzuki found a seven-nucleotide insertion between exons 13 and 14.

Patients classified as juvenile/adult have varied considerably in severity. A sizable number have been reported from Japan [12, 13, 29, 39–49]. In a Mexican family with first cousin parents, two boys and a girl had coarse features, dysostosis multiplex, shortness of stature, and impaired mental development, along with cherry red spots, corneal clouding, seizures, and hearing loss, but no hepatosplenomegaly [2]. Onset of symptoms has



Figure 100.7 The cherry red spot of the five-year-old boy.



Figure 100.8 The five-year-old had angiokeratoma of the scrotum. Histologic analysis was confirmatory.

been as early as one year of age [45] or as late as 40 years [29]. Coarse features are regularly seen, but they may be mild (Figure 100.6). Most patients have platyspondyly, but fully developed dysostosis multiplex is unusual. Hepatosplenomegaly is not common.

Neurologic features include generalized seizures and myoclonus, ataxia, and impaired mental development. Deterioration may be progressive. Deep tendon reflexes are brisk [26]. Bilateral cherry red spots are found in most patients (Figure 100.7). There may be corneal clouding, punctate lenticular opacities, and loss of visual acuity. Other patients have no neurological abnormalities.

Angiokeratomas are common [29, 45] (Figure 100.8). They are found in clusters in a distribution indistinguishable from those of Fabry disease (Chapter 88).

All patients with galactosialidosis have foam cells in the marrow and vacuolated lymphocytes in peripheral blood. Vacuoles may also be seen in Kupffer cells [50]. Pathologic features include macroscopic cerebral atrophy [50]. Membrane-bound inclusions are seen on electron microscopy of lymphocytes or skin [37], brain [51–53], peripheral Schwann cells [48, 54, 55], and in the myenteric plexus of the rectum [43, 48]. Their appearance is similar to those of GM₁ gangliosidosis and sialidosis. They may have lamellar or wavy concentric structure [52, 53]. An early infantile patient was reported in which multiple infarctions were found in the brain [56]. In another there were periventricular calcifications [30].

GENETICS AND PATHOGENESIS

The disorder, in all of its variants, is transmitted in an autosomal recessive fashion. Rates of consanguinity have been quite high. Fibroblasts of a parent were shown to have reduced amounts of mRNA for the protective protein, providing chemical evidence of heterozygosity [57]. Enzyme activity attributable to the protective protein also yielded intermediate values in fibroblasts of heterozygotes [58, 59].

The molecular defect is in the lysosomal PPCA, the existence of which became evident through studies of patients with galactosialidosis [18, 60]. This protein is normally synthesized as a 54 kDa precursor, which is modified post-translationally to 32 and 20 kDa polypeptides which proved to be the corrective factor [13, 16, 61]. Immunoprecipitation demonstrated absence of the 54, 32, and 20 kDa polypeptides in fibroblasts of patients with galactosialidosis [16]. Neuraminidase aggregates normally with β -galactosidase and protective factor in a large multimer that resists proteolytic degradation [61–64]. Neuraminidase requires protective protein for activity. The multimer aggregate correctly routes the two glycosidases to the lysosome and protects them from rapid lysosomal proteolysis. The isolation of the cDNA for the protective protein [57] and its expression in COS cells [58, 65] elucidated the structure, function, and physiology of

the protein. The sequence begins with a signal peptide that is cleaved, followed by 298 amino acids of the 32 kDa protein, which is followed by the 20 kDa protein; the two make up the 54 kDa precursor. The latter, synthesized in COS cells from the cDNA, is taken up by the fibroblasts of patients with galactosialidosis and restores activity in both enzymes [57].

Once the primary structure of the protein was known, its homology to yeast and plant serine carboxypeptidases became apparent. The protective protein was then shown to have carboxypeptidase activity [66] – and this activity is deficient in galactosialidosis. The properties of this carboxypeptidase are consistent with those of cathepsin A [58]. Site-directed mutagenesis which abolishes cathepsin activity does not interfere with protective activity; so the two functions are distinct [58]. Nevertheless, cathepsin A activity provides a simple test that is useful for heterozygote detection. The fact that the protective function and cathepsin activity are distinct provides an argument for continued diagnostic reliance on the assay of β -galactosidase and neuraminidase in leukocytes or fibroblasts [57, 58]. The three enzyme activities, cathepsin, β -galactosidase, and neuraminidase, copurify [57]. PPCA and galactosidase are found separate from the complex, but all of the neuraminidase is present in the complex [67]. PPCA functions as an intracellular transport protein [68]. It has a mannose-6-phosphate recognition marker [69]. The enzyme also has deamidase and esterase activities, and these activities are deficient in cultured cells of patients [70].

Prenatal diagnosis of an affected fetus has been accomplished by assay of β -galactosidase and neuraminidase in cultured amniocytes [32] and also by the detection of sialyloligosaccharides in amniotic fluid [71].

Failure to synthesize immunoprecipitable protective protein was found in fibroblasts of a patient with the early infantile disease [16]. There was no protective protein mRNA [57]. In contrast, in the late infantile disease there was a larger quantity of the 54 kDa precursor protein [71] and a trace amount of the mature 32 kDa protein [72]. In a patient with the juvenile/adult disease [4] a small amount of normal-sized precursor was found [71], while others in this group made precursors of various molecular sizes from 45 to 63 kDa [73]. The gene was mapped to chromosome 20 by somatic cell hybridization [60] and *in situ* hybridization [18]. There are 15 exons over 7 kb of genomic DNA [74].

Japanese patients with adult mild clinical disease were found to have a deletion of exon 7 [29, 75]. This resulted from a substitution at the donor splice site of intron 7, which causes aberrant splicing of the precursor mRNA [22]. Patients with the genotype have had relatively more severe disease with juvenile onset. The exon 7 deletion was in compound with point mutations changing glycine 49 to arginine, tryptophan 69 to arginine, and tyrosine 395 to cysteine [22, 29, 76]. Adult, milder phenotype patients with the exon 7 deletion are generally homozygous. Two patients with the late infantile disease [5, 36] were found

to have mutations for phenylalanine 412 to valine [21]. Expression in COS cells led to a precursor that was to some extent retained in the endoplasmic reticulum, which would be consistent with the finding of increased precursor and shortage of mature protein in this form [71]. A number of Caucasian patients with this form of the disease have been found to have a point mutation changing tyrosine 221 to asparagine [77]. Others had phenylalanine 112 valine 22 [78]. Early infantile patients have been found to have valine 104 methionine, leucine 208 proline, and glycine 411 serine [78].

In the PPGB gene, a novel missense mutation, one (p.G86V) was found in Portuguese patients [24], as well as a previously reported p.V104M, and two novel deletions (c.230delC and c.991-992delT) coding for non-functional proteins. A missense mutation was also found at the cathepsin A active site.

The natures of the enzymatic defects in β -galactosidase and in neuraminidase lead to the accumulation in tissues of these patients of GM₁ ganglioside [43] and other gangliosides, including GM₃, as well as sialylated storage compounds as in sialidosis [79–81]. A variety of sialyloligosaccharides is excreted in the urine. Their detection by thin layer chromatography may be useful in leading to the diagnosis [26, 58, 82]. These results are followed up with enzyme assays to establish the diagnosis, and most often the diagnosis is now made by direct assay of the activities of β -galactosidase and neuraminidase.

Clinical diagnosis has often been made by thin layer chromatography for oligosaccharides. The disease has been classified as an oligosaccharidosis. An LC MS/MS method has been developed [83] for the unique pattern found in each of the oligosaccharidoses. Capillary high-performance anion-exchange chromatography with MS has also been employed in galactosialidosis [84] to detect free oligosaccharides. O-sulfated oligosaccharides were detected and glycan products of glycosphingolipids including aldohexonic products of oligosaccharides and reducing end hexose, suggesting alternative rates of catabolism.

Oligosaccharidoses result from a deficiency in enzymes responsible for the catabolism of protein bound oligosaccharides and are typified by the accumulation of corresponding sugars in the urine. An effective screening and diagnostic tool for these disorders is by a mixed mode liquid chromatography tandem mass spectrometry assay which potentially mitigates many of the problems associated with thin layer chromatography. Each oligosaccharidosis produces a unique selected reaction monitoring fingerprint.

TREATMENT

Effective specific therapy has not been devised. The availability of animal models should permit studies on gene transfer as an approach to therapy. Bone marrow transplantation has been successful in PPCA (2/2) mice [85].

REFERENCES

- Okada S, O'Brien JS. Generalized gangliosidosis β -galactosidase deficiency. *Science* 1968; **160**: 1002.
- Goldberg MF, Cotlier E, Fischens LG *et al*. Macular cherry-red spot corneal clouding and β -galactosidase deficiency. *Arch Intern Med* 1971; **128**: 387.
- O'Brien JS, Ho MW, Veath ML *et al*. Juvenile GM₁-gangliosidosis clinical pathological chemical and enzymatic studies. *Clin Genet* 1972; **3**: 411.
- Loonen MCB, van der Lugt L, Francke CL. Angiokeratoma corporis diffusum and lysosomal enzyme deficiency. *Lancet* 1974; **ii**: 785.
- Pinsky L, Miller J, Shanfield B *et al*. GM₁ gangliosidosis in skin fibroblast culture: enzymatic differences between types 1 and 2 and observations on a third variant. *Am J Hum Genet* 1974; **26**: 563.
- Galjaard H, Hoogeveen AT, Keijzer W *et al*. Genetic heterogeneity in GM₁-gangliosidosis. *Nature* 1975; **257**: 60.
- Wenger DA, Tarby TJ, Wharton C. Macular cherry-red spots and myoclonus with dementia: coexistent neuraminidase and β -galactosidase deficiencies. *Biochem Biophys Res Commun* 1978; **82**: 589.
- Justice PM, Wenger DA, Naidu S, Rosenthal IM. Enzymatic studies in a new variant of GM₁-gangliosidosis in an older child. *Pediatr Res* 1977; **11**: 407.
- Thomas GH, Goldberg MF, Miller CS, Reynolds LW. Neuraminidase deficiency in the original patient with the Goldberg syndrome. *Clin Genet* 1979; **16**: 323.
- Lowden JA, O'Brien JS. Sialidosis: a review of human neuraminidase deficiency. *Am J Hum Genet* 1979; **31**: 1.
- Hoogeveen AT, Verheijen FW, d'Azzo A, Galjaard H. Genetic heterogeneity in human neuraminidase deficiency. *Nature* 1980; **285**: 500.
- Okada M, Inui M, Chiyo H. A case of neuraminidase deficiency associated with a partial β -galactosidase defect. Clinical biochemical and radiological studies. *Eur J Pediatr* 1981; **130**: 292.
- Hoogeveen A, d'Azzo A, Brossmer R, Galjaard H. Correction of combined β -galactosidase/neuraminidase deficiency in human fibroblasts. *Biochem Biophys Res Commun* 1981; **103**: 292.
- van Diggelen OP, Schram AW, Sinnot ML *et al*. Turnover of β -galactosidase in fibroblasts from patients with genetically different types of β -galactosidase deficiency. *Biochem J* 1981; **200**: 143.
- van Diggelen OP, Hoogeveen AT, Smith PJ *et al*. Enhanced proteolytic degradation of normal β -galactosidase in the lysosomal storage disease with combined β -galactosidase and neuraminidase deficiency. *Biochim Biophys Acta* 1982; **703**: 69.
- d'Azzo A, Hoogeveen A, Reuser AJ *et al*. Molecular defect in combined β -galactosidase and neuraminidase deficiency in man. *Proc Natl Acad Sci USA* 1982; **79**: 4535.
- Andria G, Strisciuglio P, Pontarelli G *et al*. Infantile neuraminidase and β -galactosidase deficiencies (galactosialidosis) with mild clinical courses. In: Durand P, Tettamanti G, Di Donato S (eds). *Sialidases and Sialidosis*. Milan: Edi Ermes, 1981: 379.
- Wiegant J, Galjaard NJ, Rapp AK, d'Azzo A. The gene encoding human protective protein (PPGB) is on chromosome 20. *Genomics* 1991; **10**: 345.
- Whitmore T, Day J, Albers J. Localization of the human phospholipid transfer protein gene to chromosome 20q12-q131. *Genomics* 1995; **28**: 599.
- Day J, Albers J, Lofton-Day C *et al*. Complete cDNA encoding human phospholipid transfer protein from human endothelial cells. *J Biol Chem* 1994; **269**: 9388.
- Zhou XY, Galjart NJ, Willemsen R *et al*. A mutation in a mild form of galactosialidosis impairs dimerization of the protective protein and renders it unstable. *EMBO J* 1991; **10**: 4041.
- Shimmoto M, Fukuhara Y, Itoh K *et al*. Protective protein gene mutations in galactosialidosis. *J Clin Invest* 1993; **91**: 2393.
- d'Azzo A, Bonten E. Molecular mechanisms of pathogenesis in a glycosphingolipid and a glycoprotein storage disease. *Biochem Soc Trans* 2010; **38**: 1453.
- Coutinho M, Lacerda L, Macedo-Ribeiro S *et al*. Lysosomal multienzymatic complex-related diseases: a genetic study among Portuguese patients. *Clin Genet* 2011; epub ahead of print.
- Gravel RA, Lowden JA, Callahan JW *et al*. Infantile sialidosis. A phenocopy of type 1 GM₁ gangliosidosis distinguished by genetic complementation and urinary oligosaccharides. *Am J Hum Genet* 1979; **31**: 669.
- Suzuki Y, Nanba E, Tsuji A *et al*. Clinical and genetic heterogeneity in galactosialidosis. *Brain Dysfunct* 1988; **1**: 285.
- Okada S, Sugino H, Kato T *et al*. A severe infantile sialidosis (β -galactosidase- γ -neuraminidase deficiency) mimicking GM₁-gangliosidosis type 1. *Eur J Pediatr* 1983; **140**: 295.
- Yamano T, Dezawa T, Koike M *et al*. Ultrastructural study on a severe infantile sialidosis (β -galactosidase-a-neuraminidase deficiency). *Neuropediatrics* 1985; **16**: 109.
- Takano T, Shimmoto M, Fukuhara Y *et al*. Galactosialidosis: clinical and molecular analysis of 19 Japanese patients. *Brain Dysfunct* 1991; **4**: 271.
- Zammarchi E, Donati MA, Marrone A *et al*. Early infantile galactosialidosis: clinical biochemical and molecular observations in a new patient. *Am J Med Genet* 1996; **64**: 453.
- Claeys M, Van Der Hoeven M, de Die-Smulders C *et al*. Early-infantile type of galactosialidosis as a case of heart failure and neonatal ascites. *J Inher Metab Dis* 1999; **22**: 666.
- Kleijer WJ, Hoogeveen A, Verheijen FW *et al*. Prenatal diagnosis of sialidosis with combined neuraminidase and β -galactosidase deficiency. *Clin Genet* 1979; **16**: 60.
- Landau D, Zeigler M, Shinwell E *et al*. Hydrops fetalis in four siblings caused by galactosialidosis. *Isr J Med Sci* 1995; **31**: 321.
- Tekinalp G, Aliefendioglu D, Yuce A *et al*. A case with early infantile form of galactosialidosis with unusual haematological findings. *J Inher Metab Dis* 1999; **22**: 668.
- Olçay L, Gumruk F, Boduroglu K *et al*. Anaemia and thrombocytopenia due to haemophagocytosis in a 7-month-old boy with galactosialidosis. *J Inher Metab Dis* 1998; **21**: 679.
- Strisciuglio P, Sly W, Dodson WE *et al*. Combined deficiency of β -galactosidase and neuraminidase: natural history of the

- disease in the first 18 years of an American patient with late infantile onset form. *Am J Med Genet* 1990; **37**: 573.
37. Chitayat D, Applegarth DA, Lewis J *et al.* Juvenile galactosialidosis in a white male: a new variant. *Am J Med Genet* 1988; **31**: 887.
 38. Richard C, Tranchemontagne J, Elsliger M *et al.* Molecular pathology of galactosialidosis in a patient affected with two new frameshift mutations in the cathepsin A/protective protein gene. *Hum Mutat* 1998; **11**: 461.
 39. Orii T, Minami R, Sukegawa K *et al.* A new type of mucopolipidosis with β -galactosidase deficiency and glycopeptiduria. *Tohoku J Exp Med* 1972; **107**: 303.
 40. Yamamoto A, Adachi S, Kawamura S *et al.* Localized β -galactosidase deficiency. Occurrence in cerebellar ataxia with myoclonus epilepsy and macular cherry red-spot. A new variant of GM₁-gangliosidosis. *Arch Intern Med* 1974; **134**: 627.
 41. Suzuki Y, Fukuoka K, Sakuraba H. β -Galactosidase neuraminidase deficiency with cerebellar ataxia and myoclonus. In: Sobue I (ed.). *Spinocerebellar Degenerations*. Tokyo: University of Tokyo, 1980: 339.
 42. Matsuo T, Egawa I, Okada S *et al.* Sialidosis type 2 in Japan. Clinical study in two siblings cases and review of literature. *J Neurol Sci* 1983; **58**: 45.
 43. Yoshino H, Miyashita K, Miyatani N *et al.* Abnormal glycosphingolipid metabolism in the nervous system of galactosialidosis. *J Neurol Sci* 1990; **97**: 53.
 44. Tsuiji S, Yamada T, Ariga T *et al.* Carrier detection of sialidosis with partial β -galactosidase deficiency by the assay of lysosomal sialidase in lymphocytes. *Ann Neurol* 1984; **15**: 181.
 45. Suzuki Y, Sakuraba H, Yamanaka T *et al.* Galactosialidosis: a comparative study of clinical and biochemical data on 22 patients. In: Arima M, Suzuki Y, Yabuuchi H (eds). *The Developing Brain and Its Disorders*. Tokyo: University of Tokyo, 1984: 161.
 46. Suzuki Y, Nakamura N, Fukuoka K *et al.* β -Galactosidase deficiency in juvenile and adult patients. *Hum Genet* 1977; **36**: 219.
 47. Tsuiji S, Yamada T, Tsutsumi A, Miyatake T. Neuraminidase deficiency and accumulation of sialic acid in lymphocytes in adult type sialidosis with partial β -galactosialidase. *Ann Neurol* 1982; **11**: 541.
 48. Miyatake T, Atsumi T, Obayashi T *et al.* Adult type neuronal storage disease with neuraminidase deficiency. *Ann Neurol* 1979; **6**: 232.
 49. Kuriyama M, Miyatake T, Owada M, Kitagawa T. Neuraminidase activities in sialidosis and mucopolipidosis. *J Neurol Sci* 1982; **54**: 181.
 50. Berard-Badier M, Adechy-Benkoel L, Chamlian A *et al.* Etude ultrastructurale du parenchyme hépatique dans les mucopolysaccharidoses. *Path Biol (Paris)* 1970; **18**: 117.
 51. Giljaard H, Hoogveen A, Verheijen FW *et al.* Relationship between clinical, biochemical and genetic heterogeneity in sialidase deficiency. In: Tettamanti G, Durand P, Di Donato S (eds). *Sialidoses and Sialidoses*. Milan: Edi Ermes, 1981: 371.
 52. Oyanagi K, Ohama E, Miyashita K *et al.* Galactosialidosis: neuropathological findings in a case of the late-infantile type. *Acta Neuropathol* 1991; **82**: 331.
 53. Amano N, Yokoi S, Akagi M *et al.* Neuropathological findings of an autopsy case of adult β -galactosidase and neuraminidase deficiency. *Acta Neuropathol* 1983; **61**: 283.
 54. Kobayashi T, Ohta M, Goto I *et al.* Adult type mucopolipidosis with β -galactosidase and sialidase deficiency. *J Neurol* 1979; **221**: 137.
 55. Ishibashi A, Tsuboi R, Shinmei M. β -Galactosidase and neuraminidase deficiency associated with angiokeratoma corporis diffusum. *Arch Dermatol* 1984; **120**: 1344.
 56. Nordborg C, Kyllerman M, Conradi N, Mansson J. Early infantile galactosialidosis with multiple brain infarctions: morphological neuropathological and neurochemical findings. *Acta Neuropathol* 1997; **93**: 24.
 57. Galjart NH, Gillems N, Harris A *et al.* Expression of cDNA encoding the human 'protective protein' associated with lysosomal β -galactosidase and neuraminidase: homology to yeast proteases. *Cell* 1988; **54**: 755.
 58. Galjart NH, Morreau H, Willemsen R *et al.* Human lysosomal protective protein has cathepsin A-like activity distinct from its protective function. *J Biol Chem* 1991; **226**: 14 754.
 59. Itoh K, Takiyama N, Nagao Y *et al.* Acid carboxypeptidase deficiency in galactosialidosis. *Jpn J Hum Genet* 1991; **36**: 171.
 60. Mueller OT, Henry WM, Haley LL *et al.* Sialidosis and galactosialidosis: chromosomal assignment of two genes associated with neuraminidase-deficiency disorders. *Proc Natl Acad Sci USA* 1986; **83**: 1817.
 61. Hoogveen AT, Verheijen FW, Galjaard H. The relation between human lysosomal β -galactosidase and its protective protein. *J Biol Chem* 1983; **258**: 12 143.
 62. Suzuki Y, Sakuraba H, Hayashi K *et al.* β -Galactosidase-neuraminidase deficiency: restoration of β -galactosidase activity by protease inhibitors. *J Biochem* 1981; **90**: 271.
 63. Verheijen FW, Bossmmer R, Galjaard H. Purification of acid β -galactosidase and acid neuraminidase from bovine testis: evidence for an enzyme complex. *Biochem Biophys Res Commun* 1982; **108**: 868.
 64. Verheijen FW, Palmeri S, Hoogveen AT, Galjaard H. Human placental neuraminidase activation stabilization and association with β -galactosidase and its protective protein. *Eur J Biochem* 1985; **149**: 315.
 65. Bonten E, van der Spoel A, Fornerod M *et al.* Characterization of human lysosomal neuraminidase defines the molecular basis of the metabolic storage disorder sialidosis. *Genes Dev* 1996; **10**: 3156.
 66. Tranchemontagne J, Michaud L, Potier M. Deficient lysosomal carboxypeptidase activity in galactosialidosis. *Biochem Biophys Res Commun* 1990; **168**: 22.
 67. Pshezhetsky A, Potier M. Association on N-acetylgalactosamine-6-sulfate sulfatase with the multienzyme lysosomal complex of β -galactosidase cathepsin A and neuraminidase. *J Biol Chem* 1996; **271**: 28 359.
 68. van der Spoel A, Bonten E, d'Azzo A. Transport of human lysosomal neuraminidase to mature lysosomes requires protective protein/cathepsin A. *EMBO J* 1998; **17**: 1588.
 69. Morreau H, Galjart NJ, Willemsen R *et al.* Human lysosomal protective protein. Glycosylation intracellular transport

- and association with β -galactosidase in the endoplasmic reticulum. *J Biol Chem* 1992; **267**: 17949.
70. Kase R, Itoh K, Takiyama N *et al*. Galactosialidosis: simultaneous deficiency of esterase carboxy-terminal deamidase and acid carboxypeptidase activities. *Biochem Biophys Res Commun* 1990; **172**: 1175.
71. Sewell AC, Pontz BF. Prenatal diagnosis of galactosialidosis. *Prenat Diagn* 1988; **8**: 151.
72. Strisciuglio P, Parenti G, Giudice C *et al*. The presence of a reduced amount of 32-kd 'protective' protein is a distinct biochemical finding in late infantile galactosialidosis. *Hum Genet* 1988; **80**: 304.
73. Nanba ET, Tsuji A, Omura K, Suzuki Y. Galactosialidosis: molecular heterogeneity in biosynthesis and processing of protective protein for β -galactosidase. *Hum Genet* 1988; **80**: 329.
74. Shimmoto M, Nakahori Y, Matsushita I *et al*. A human protective protein gene partially overlaps the gene encoding phospholipids transfer protein on the complementary strand of DNA. *Biochem Biophys Res Commun* 1996; **220**: 802.
75. Shimmoto M, Takano T, Fukuhara Y *et al*. Japanese-type adult galactosialidosis. A unique and common splice junction mutation causing exon skipping in the protective protein carboxypeptidase gene. *Proc Jpn Acad* 1990; **66**: 217.
76. Fukuhara Y, Takano T, Shimmoto M *et al*. A new point mutation of protective protein gene in two Japanese siblings with juvenile galactosialidosis. *Brain Dysfunct* 1992; **5**: 319.
77. Zhou XY, Willemsen R, Gillemans N *et al*. Common point mutations in four patients with the late infantile form of galactosialidosis. *Am J Hum Genet* 1993; **53**: 966A.
78. Zhou XY, van der Spoel A, Rottier R *et al*. Molecular and biochemical analysis of protective protein/cathepsin A mutations: correlation with clinical severity in galactosialidosis. *Hum Mol Genet* 1996; **5**: 1977.
79. van Pelt J, Bakker HD, Kamerling JP, Vliegthart JFG. A comparative study of sialyloligosaccharides isolated from sialidosis and galactosialidosis in urine. *J Inher Metab Dis* 1991; **14**: 730.
80. van Pelt J, van Kuik JA, Kamerling JP *et al*. Storage of sialic acid containing carbohydrates in the placenta of a human galactosialidosis fetus. Isolation and structural characterization of 16 sialyloligosaccharides. *Eur J Biochem* 1988; **177**: 327.
81. van Pelt J, Kamerling JP, Vliegthart JFG. A comparative study of the accumulated sialic acid-containing oligosaccharides from cultured human galactosialidosis and sialidosis fibroblasts. *Clin Chim Acta* 1988; **174**: 325.
82. Sewell AC. An improved thin layer chromatographic method for urinary oligosaccharide screening. *Clin Chim Acta* 1979; **92**: 411.
83. Sowell J, Wood T. Towards a selected reaction monitoring mass spectrometry fingerprint approach for the screening of oligosaccharidoses. *Anal Chim Acta* 2011; **686**: 102.
84. Bruggink C, Poorthuis BJ, Piraud M *et al*. Glycan profiling of urine, amniotic fluid and ascitic fluid from galactosialidosis patients reveals novel oligosaccharides with reducing end hexose and aldohexonic acid residues. *FEBS J* 2010; **277**: 2970.
85. Zhou XY, Morreau H, Rottier R *et al*. Mouse model for the lysosomal disorder galactosialidosis and correction of the phenotype with over-expressing erythroid precursor cells. *Genes Dev* 1995; **9**: 2623.

Metachromatic leukodystrophy

Introduction	760	Treatment	764
Clinical abnormalities	761	References	765
Genetics and pathogenesis	763		

MAJOR PHENOTYPIC EXPRESSION

Delay or deterioration in walking, progressive neurodegenerative disease, optic atrophy, and grayish discoloration of the retina, symmetrical decrease in the density of cerebral white matter, elevated cerebrospinal fluid protein, increased excretion of urinary sulfatide, and deficient activity of arylsulfatase A.

INTRODUCTION

Metachromatic staining of the brain in neurodegenerative disease was reported as early as 1910 by Perusini and by Alzheimer [1, 2] in studies of adults. The classic late infantile form of metachromatic leukodystrophy (MLD) was first reported by Greenfield [3] in 1933. In 1925, Scholz [4] published a detailed clinical pathologic study of juvenile or childhood-onset leukodystrophy, and 34 years later, Peiffer [5] demonstrated that the neural tissues of Scholz's frozen sections stained metachromatically. The metachromasia results from the accumulation of sulfatides, and this was discovered independently in 1958 by Jatzkewitz [6] and Austin [7]. It was Austin and his colleagues [8] who found the defective activity of arylsulfatase A. Mehl and Jatzkewitz [9] demonstrated defective activity against cerebroside sulfate, the material that accumulates in MLD (Figure 101.1).

Arylsulfatase A (ASA) is the enzyme responsible for desulfation of the lipid component myelin. In MLD, there is intralysosomal storage of glycosphingolipid sulfatide, which leads to progressive neurologic disease [10].

The sulfatase enzyme is heat labile. A heat stable factor that increases activity several fold is known as saposin B, and in rare instances MLD results from defective activity of this protein, and arylsulfatase activity is normal [11, 12]. Deficiency of the heat stable factor causes deficiency of the enzymatic hydrolysis of sulfatide [13, 14]. Other variants with clinical MLD have signs of mucopolysaccharidosis and have been found to have multiple sulfatase deficiency (Chapter 102).

The gene for arylsulfatase A has been localized distal to band q13 on chromosome 22 [15], and it has been cloned and sequenced [16]. A number and variety of mutations have been elucidated. In general, patients with late infantile MLD have null mutations leading to absence of enzyme activity and immunoreactive enzyme either in patient cells or when the gene is introduced into animal cell-line expression systems [17]. Mutations which express small amounts of cross-reacting material (CRM) and active enzyme are found on at least one allele in juvenile onset and adult disease.

The diagnosis of MLD is complicated by the fact that there is a benign pseudodeficiency allele for arylsulfatase A,

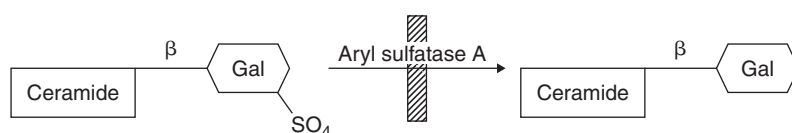


Figure 101.1 The arylsulfatase A reaction, site of the defect in metachromatic leukodystrophy. CER, ceramide; GAL, galactose; SO₄, sulfate. A number of other sulfatides, such as lactosylceramide sulfate, as well as the galactosylceramide sulfate shown, are natural substrates for the enzyme.

which in homozygotes leads to low arylsulfatase A activity and no clinical abnormalities [18, 19]. Thus, not only can patients with MLD have normal activity of arylsulfatase A (when the deficiency is in saposin B), but patients with little or no activity of arylsulfatase A may not have MLD (when they have the pseudodeficiency allele in homozygosity).

CLINICAL ABNORMALITIES

MLD has been divided into three or five subgroups on the basis of the age of onset and rapidity of neurologic degeneration. In one classification, the proportions were the late-infantile (50–60 percent of patients); juvenile (20–30 percent); and adult (15–20 percent) [20]. As in the case of many genetic diseases, the advent of molecular biology may make all of these classifications obsolete, but it currently remains useful to distinguish at least the infantile and adult phenotypes. The classic late infantile disease (Figures 101.2 and 101.3) begins before 30 months of life and is progressive to death in one to seven years. MLD accounts for approximately 8.2 percent of the leukodystrophies of children [21]. The first manifestations are loss of acquired motor skills, especially walking, which becomes unsteady. Examination at this time reveals hypotonia, and often a pronounced genu recurvatum. Deep tendon reflexes are diminished or even absent, indicative of neuropathy. In some patients, walking is delayed, and some never learn to walk [22, 23], but most learn to walk unassisted and to speak short sentences, and then these skills deteriorate. Intercurrent infection may be followed by ataxia and weakness, which may disappear, but reappear later. The initial presentation with hypotonia and reflex changes may suggest a myopathy or peripheral neuropathy [24–26]. There may be intermittent severe pains in the legs



Figure 101.2 SAS: A 24-month-old infant with metachromatic leukodystrophy was markedly hypotonic and assumed a frog-leg position. He had lost milestones achieved. He had markedly diminished deep tendon reflexes. Arylsulfatase A activity was <0.1 nmol/mg per hour.



Figure 101.3 SAS: The closed eyelids symbolize loss of contact with surroundings. Cerebrospinal fluid (CSF) protein was 80 mg/dL. Nerve conduction velocity was diminished.

[25]. Magnetic resonance imaging (MRI) [27] revealed diffuse enhancement cranial nerve and cauda equina nerve roots in a patient with infantile metachromatic leukodystrophy [28]. Hagberg [25] has viewed the progression of the disease in four stages, the initial picture representing stage I. In stage II, the patient is no longer able to stand, but can sit. There is ataxia and truncal titubation. Speech deteriorates and is dysarthric or aphasic, and mental function regresses. Muscle tone is increased in the legs and deep tendon reflexes are exaggerated. Ocular nystagmus develops, and ophthalmoscopy reveals optic atrophy and a grayish discoloration of the retina and macula, sometimes with a central red spot reminiscent of Tay-Sachs disease [26] (Chapter 90). In stage III, the patient develops spastic quadriplegia and is confined to bed. There may be decerebrate or decorticate rigidity or dystonic movements. Seizures develop in about a third of patients [25]. Pharyngeal muscle coordination is lost, and there is difficulty with feeding and with the airway. Mental deterioration continues, and speech is lost. The child may continue to respond to parents and smile. In stage IV, contact is lost. The patient is blind and cannot swallow. Tube feeding is required. Death results usually from pneumonia. In 29 Brazilian patients with a spectrum of phenotypes, the first initial manifestation of disease was disturbance of gait or other motor abnormality (72.7 percent) in the late infantile form and behavioral or cognitive abnormalities (50 percent) in the juvenile form [29].

Juvenile MLD has been the designation of patients where initial presentation was between four and 16 years of age, often with a decrease in school performance in the first or second grade, sometimes with unusual behavior [30]. The patient may appear confused or to be daydreaming. Some have had dementia, psychosis, or emotional illness. Younger patients may present with clumsiness of gait, as in the infantile patients [28]. Muscular rigidity, postural abnormalities, and ataxia may occur. Within a year of onset, the patient is unable to walk. Urinary incontinence may occur early. Progression is to stages III and IV, as in the late infantile disease. It is clear that patients within this group may have phenotypes overlapping those of younger and older patients; the distinction may be artificial. In fact, instances have been described of siblings in the same family with juvenile and adult disease [31–35]. Some unusual visceral presentations have been with acute cholecystitis [31], chronic hemorrhagic pancreatitis [36], abdominal mass [37], or gastrointestinal bleeding [38]. There may be polyps in the intestine and in the gallbladder [39].

Adult MLD refers to patients presenting after puberty [40]. Onset may be as young as 15 years of age [41] or as late as 62 years. Survival may be for five or ten years or longer. Symptomatology is largely psychiatric. The recognition of these patients is a strong argument for neuroimaging studies in psychiatric patients [42]. Dementia may be manifest in loss of memory or decrease in intellectual ability. Psychotic changes may be those of schizophrenia. There may be emotional lability, anxiety, or apathy. Visual–spatial discrimination may be impaired. Auditory hallucinations and delusions were reported in 18 and 27 percent of patients, and psychosis in 53 percent [43]. Depression and chronic alcoholism have been observed.

Motor disturbances may develop with clumsiness of gait and dysarthria. Muscle tone increases and deep tendon reflexes are brisk. Some develop ataxia and some have Parkinson-like features. In some patients, the initial manifestations are those of peripheral neuropathy [44, 45]. Dystonic movements may develop. Degeneration progresses to spastic tetraparesis, bulbar involvement, and decorticate posturing. Optic atrophy and nystagmus are found. There may be seizures. Ultimately, the patient is blind, mute, and unresponsive. Gross Motor Function Classification (GMFC-MLD) has been standardized as a tool for the assessment of gross motor function in MLD, description of the natural course of the disease, and evaluation of therapy [46].

Patients with MLD as a result of deficiency of the cerebroside sulfatase activator, saposin B, have generally presented as juvenile MLD [11, 12, 47]. In one patient, onset was at 48 years with intellectual deterioration, apathy, and withdrawal [48]. Each was recognized initially on the basis of an MLD phenotype with normal activity of arylsulfatase A [13].

The clinical laboratory evaluation of patients with established MLD is notable for elevation of the concentration of protein in the cerebrospinal fluid. The level

may be normal early in infantile disease, but it rises progressively to levels of 100 mg/dL or higher. This is true also for the younger-onset juvenile patients; while later-onset juvenile and adult-onset patients usually have normal levels of protein, though there have been a few with elevated concentrations [43].

The electroencephalograph (EEG) may be abnormal, especially in those with seizures [48–51]. There may be diffuse slowing or spike discharges, often focal. Noise may induce a marked startle response. The EEG tends to be normal in the adult-onset patient [51].

Motor nerve conduction is slowed [28]. These abnormalities have been demonstrated in presymptomatic patients, indicating the presence of peripheral neuropathy

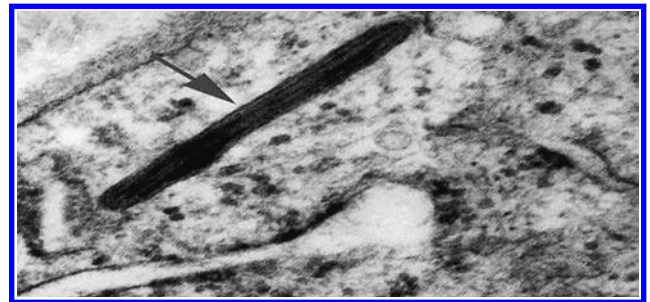


Figure 101.4 Electron microscopy of biopsied sural nerve indicating the dense inclusion body. These inclusions have been called zebra bodies because of the stripes.

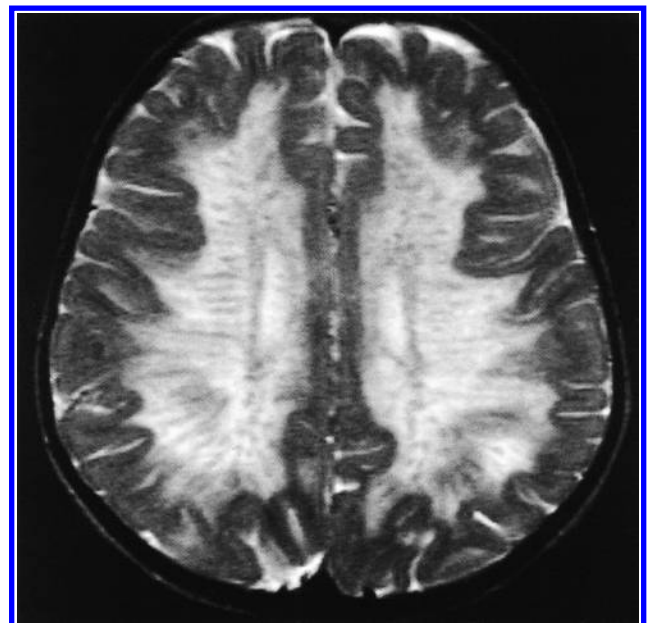


Figure 101.5 Magnetic resonance image of the brain of a five-year-old female with metachromatic leukodystrophy. There was diffuse high T_2 signal throughout the white matter in a sunray appearance. She had regressed developmentally and had diminished deep tendon reflexes.

well before the onset of symptoms. Delay may be evident in afferent nerves before that of efferent nerves [52–55]. There may also be abnormalities in brainstem auditory evoked responses (BAER), visual evoked responses, or somatosensory responses. Abnormalities in BAER may be evident when peripheral nerve conduction is unimpaired [56]. Biopsy of peripheral nerve reveals the characteristic inclusion bodies (Figure 101.4). Neuroimaging by computed tomography (CT) or magnetic resonance (MR) is consistent with loss of myelin and increase in water. Low density on CT and hyperintense T_2 images on MRI are visible in periventricular white matter indicative of leukodystrophy (Figure 101.5) [57, 58]. Later there is evident atrophy. Proton magnetic resonance spectroscopy (MRS) reveals reduction in N-acetylaspartate and increase of myoinositol, a glial marker [59]. N-acetylaspartate (NAA) is a marker of loss for neurons and axons. It can be measured *in vivo* by proton MRS. In MLD, the NAA signal was reported to decrease during progression of the disease [60]. Screening test for lysosomal storage diseases was successfully carried out using vacuolated peripheral lymphocytes. Lysosomal protein profiling has been employed [61] via high throughput assay of dried blood spots. Positive identification was obtained in 99 percent of patients, including MLD.

GENETICS AND PATHOGENESIS

MLD is inherited in an autosomal recessive fashion. Multiple affected siblings of both sexes and normal parents have often been observed, and consanguinity has been noted early [62]. The incidence of the late infantile form has been estimated at one in 40,000 in Sweden [63]; the juvenile form is about four times less common. An unusually high incidence of late infantile MLD of one in 75 live births was reported in an isolate of Habbani Jews [64].

The molecular defect is in arylsulfatase A [8], which acts in tissues as the cerebroside sulfatase (Figure 101.1). This acidic glycoprotein enzyme is synthesized as a 62-kDa precursor protein, and then translocated via a mannose-6-phosphate receptor to the lysosome as 57–62 kDa forms [65]. The deficiency has been demonstrated in many different tissues, including brain, cultured fibroblasts, leukocytes, and urine [66–70]. The enzyme activity is usually measured against artificial substrate, such as p-nitrocatechol sulfate; the assay usually reveals some residual activity. Immunochemical studies have revealed CRM-positive and CRM-negative examples of late infantile MLD [71, 72]. There is evidence of rapid degradation of synthesized enzyme in juvenile and adult-onset MLD. Studies of the degradation of cerebroside sulfate in intact fibroblasts have yielded correlations between the effectiveness of the cells in catabolizing sulfatide and the age or sex and severity of the clinical phenotype [33, 73]. Arylsulfatase A activity is also defective, along with

those of other sulfatases in multiple sulfatase deficiency (Chapter 102).

Arylsulfatase A activity is normal in patients with MLD that results from the activator saposin B [14], but cultured cells from these patients fail to degrade added sulfatide. Addition of purified activator protein corrects this defective behavior, and immunologic study reveals an absence of CRM against saposin B protein [47, 74, 75].

Pseudodeficiency of arylsulfatase A was first identified through the testing of clinically normal relatives of patients with MLD [76–78]. These individuals have a pseudodeficiency gene (pd), which leads to arylsulfatase A activity of 5–15 percent of normal [71]. They do not have sulfatiduria or storage of metachromatic material. The protein is kinetically normal, but smaller in size, and it lacks a glycosyl subunit.

The gene for arylsulfatase A is on chromosome 22 at q13 [15, 78–80]. The saposin B gene is on chromosome 10 [81, 82]. The arylsulfatase A gene consists of eight exons in a small 3.2-kb coding area [83]; the mRNA is 2.1 kb. A large number of mutations has been found for arylsulfatase A [17, 84, 85]. A common polymorphism leads to an enzyme with perfectly normal activity and a threonine to serine change in exon 7 [17]. In late infantile MLD, the mutant alleles are sometimes referred to as I-type mutations [17]. More often these are now referred to as null alleles, which code for no enzyme activity. Two splice-site mutations have been identified that lead to this phenotype. They include the G609A transition that destroys the splice donor site of exon 2 by changing the exon–intron boundary from AGgt to AGat [17]. This common mutation in Europeans has also been seen in Arabs. Another was a G2195A transition at the splice-recognition site between exon 7 and the next intron [86]. In addition, an 11-bp deletion in exon 8, which causes a frame shift, was also found in this phenotype [87]. Point mutations have also been found in this phenotype, including a glycine 99 to aspartic acid change in exon 2 [88], common in Japan, and a glycine 245 to arginine change in exon 4 [89]. Other common mutations among Europeans are P426L [17] and I79S [84]. Mutations, such as the G-to-A transition in exon 2 which results in a change from glycine 99 to aspartic acid [90] and the proline 426 change to leucine have been referred to as type A mutations and in the homozygous situation lead to adult-onset MLD. They are now referred to as R alleles and they code for some residual activity. In 16 Saudi patients with MLD, seven patients had arylsulfatase A-deficiency and had sphingolipid activator deficiency. The later four were homozygous for a g.722G>C transversion which led to a p.C241S previously reported mutation [91] and four have sphingolipid. Compound heterozygosity for the mutation (p.G99D and p.T409I0) was found in a Japanese female with behavioral abnormalities [92]. Nine pathogenic mutations were found in 13 Indian patients (65 percent), five of them novel. The most common mutation was c.459+16>A [93]. Compounds of A- and I-type mutations have been found in juvenile onset patients [17]. In a

Korean boy who could not walk at 12 months and died at nine years, a novel splicing mutation (c.1101+1G>T) in intron 6 was found on one allele, as well as a missense mutation in exon 2 (c.296G>A; p.Gly99Asp) [94].

The pseudodeficiency enzyme is 2–4 kDa smaller than normal enzyme. This is a result of a point mutation (asparagine to serine) at the C-terminal glycosylation site leading to loss of an oligosaccharide side chain [95]. The Pro426Leu mutation, second most common in Europeans, codes for an enzyme that is synthesized normally and targeted normally to the lysosomes, but there it is promptly degraded [96]. Two R alleles are usually found in the adult-onset disease [17, 82, 97]. Patients with I179S on one allele usually begin with psychiatric manifestations, while those carrying P426L usually begin with a neurologic picture [98].

The molecular biology of the saposin B activator has also been clarified. A prosaposin precursor gene directs the synthesis of a precursor protein from which the individual saposins are derived [99]. Mutations have been identified including a C-to-T transition leading to a substitution of isoleucine for threonine that eliminates a glycosylation site with a neighboring asparagine [100] in the original family of Shapiro and colleagues [11].

Heterozygote detection has been accomplished by assay of arylsulfatase A activity in leukocytes and fibroblasts [101]. Overlap with the normal range made the designation of noncarrier less reliable. The pseudodeficiency allele is common [14] and this may even suggest that a relative is affected. Testing with sulfatide-loaded fibroblasts may be required for resolution [101]; molecular detection of the pseudodeficiency pd allele will also resolve this. The pd allele causes two A-to-G mutations, changing arginine 352 to serine, with loss of a glycosylation site, and the change of a polyadenylation signal. In families in which the mutation is known, this information can be used for heterozygote detection. Searching for the most common splice-site mutation in late infantile MLD may be particularly useful.

It may also be employed for prenatal diagnosis. So far, enzyme assay has been employed for this purpose [70, 102, 103]. Prenatal diagnosis has been accomplished with cultured amniocytes and chorionic villus material. Here, too, the pseudodeficiency allele is a problem that must be recognized and dealt with. Sulfatide loading is usually helpful.

The consequence of cerebroside sulfatase deficiency is the accumulation of sulfatides in tissues, notably the cerebral white matter [6, 7, 104]. In addition to sulfatide, lipid lysosulfatide which has been found in MLD, is cytotoxic in cell culture. High performance liquid chromatography revealed accumulation of lysosulfatide in the brain arylsulfatase A of null mutant mice [105].

The accumulated sulfatide leads to decreased content of cerebroside and the other lipid components of myelin. Sulfatide is also found in increased quantity in the urine [106]. The amounts may be 100–200 times the normal level [107, 108]. This property has sometimes been employed to identify patients for testing for saposin B deficiency in those

with clinical MLD and normal arylsulfatase A activity. Patients with pseudodeficiency do not have sulfatiduria [109].

The demyelination that characterizes the disease is doubtless a consequence of the accumulated sulfatide. Neuropathologic changes have been seen even in fetuses at the fifth month of presentation. In particular, the oligodendroglia and Schwann cells appear to be targeted.

TREATMENT

Treatment of patients with MLD has been largely supportive, including conventional treatment of seizures, prevention of contractures with muscle relaxants, physical therapy, and family support [110]. Vigabatrin may be useful in reducing spasticity [111].

Bone marrow transplantation has been employed in a number of patients [112–117]. Normal levels of circulating arylsulfatase A have been achieved, and the clinical course has seemed to slow, particularly as compared with that of an affected sibling [113, 114]. It appears most useful in presymptomatic or early symptomatic patients. It may even accelerate progression in rapidly deteriorating patients [7, 110]. In ten families in which presymptomatic diagnosis was made because a previous sibling had had disease, there was successful engraftment in each [116]. Best results were in juvenile and adolescent forms. Adults with psychiatric disease may benefit from transplantation [116]. Transplantation is currently not recommended for symptomatic early onset forms of the disease [117].

Transplantation of bone marrow cells expressing the homeobox B4 in mice with MLD led to immunohistologic evidence of enzyme in microglia and improvement in ability to walk [118]. Hematopoietic stem-cell transplantation was without clinical improvement in MLD despite chromosomal and normal leukocyte arylsulfatase activity [118]. The results of allogeneic cell therapy in five patients with adult-onset MLD were reported to be poor [119]. The outcome of unrelated umbilical cord blood transplantation in three siblings with juvenile MLD was reported to be an inhibition of progression and stabilization of disease [120]. Enzyme replacement therapy (ERT), which has been successful in many lysosomal storage diseases notably Gaucher disease (Chapter 93), has not been successful in disorders such as MLD with prominent cerebral manifestations because of the efficiency of the blood–brain barrier. Infusion of recombinant human arylsulfatase A (rhASA) by an implanted miniature pump into the cerebral ventricular fluid of knockout mouse models of MLD indicated penetration of brain parenchyma and targeting to lysosomes. Histological examination indicated reversal of lysosomal storage, and correction of ataxic gait [10]. In an *in vitro* model system, porcine brain capillary endothelial cells were developed as an approach to increasing blood to brain transfer of sulfatase and improve therapeutic efficacy of enzyme replacement therapy [121].

REFERENCES

1. Perusini G. Über klinisch und histologisch eigenartige psychische Erkrankungen des späteren Lebensalters. *Nissl-Alzheimer's Histol Histopathol Arb* 1910; **3**: 297.
2. Alzheimer A. Beiträge zur Kenntnis der pathologischen Neurologia und ihrer Beziehung zu den Abbauvorgängen im Nervengewebe. *Nissl-Alzheimer's Histol Histopathol Arb* 1910; **3**: 493.
3. Greenfield JG. A form of progressive cerebral sclerosis in infants associated with primary degeneration of the interfascicular glia. *J Neurol Psychopathol* 1933; **13**: 289.
4. Scholz W. Klinische, pathologisch-anatomische und erbliche Untersuchungen bei familiärer, diffuser Hirnsklerose im Kindesalter. *Z Gesamte Neurol Psychiatr* 1925; **99**: 42.
5. Peiffer J. Über die metachromatischen Leukodystrophien (Typ Scholz). *Arch Psychiatr Nervenkr* 1959; **199**: 386.
6. Jatzkewitz H. Zwei Typen von Cerebrosid-schwefelsäuresog. 'Pralipoide' und Speichersubstanzen bei der Leukodystrophie, Typ Scholz (metachromatisch Form der diffusel Sklerose). *Z Physiol Chem* 1958; **311**: 279.
7. Austin J. Metachromatic sulfatides in cerebral white matter and kidney. *Proc Soc Exp Biol Med* 1958; **100**: 361.
8. Austin JH, Balasubramanian AS, Pattabiraman TN et al. A controlled study of enzymatic activities in three human disorders of glycolipid metabolism. *J Neurochem* 1963; **10**: 805.
9. Mehl E, Jatzkewitz H. Evidence for the genetic block in metachromatic leukodystrophy (ML). *Biochem Biophys Res Commun* 1965; **19**: 407.
10. Stroobants S, Gerlach D, Matthes F et al. Intracerebroventricular enzyme infusion corrects central nervous system pathology and dysfunction in a mouse model of metachromatic leukodystrophy. *Hum Mol Genet* 2011; **20**: 2760–9.
11. Shapiro LJ, Aleck KA, Kaback MM et al. Metachromatic leukodystrophy without arylsulfatase A deficiency. *Pediatr Res* 1979; **13**: 1179.
12. Hahn AF, Gordon BA, Feleki V et al. A variant form of metachromatic leukodystrophy without arylsulfatase deficiency. *Ann Neurol* 1989; **12**: 33.
13. Stevens RL, Fluharty AL, Kihara H et al. Cerebroside sulfatase activator deficiency induced metachromatic leukodystrophy. *Am J Hum Genet* 1981; **33**: 900.
14. Wenger DA, Inui K. Studies on the sphingolipid activator protein for the enzymatic hydrolysis of GM₁ ganglioside and sulfatide. In: Brady RO, Barranger JA (eds). *The Molecular Basis of Lysosomal Storage Disorders*. New York: Academic Press, 1984: 61.
15. Geurts van Kessel AHM, Westerveld A, de Groot PG et al. Regional localization of the genes coding for human AC02, ARSA, and NAGA on chromosome 22. *Cytogenet Cell Genet* 1980; **28**: 169.
16. Stein C, Gieselmann V, Kreysing J et al. Cloning and expression of human arylsulfatase A. *Biol Chem* 1989; **264**: 1252.
17. Polten A, Fluharty CB, Kappler J et al. Molecular basis of different forms of metachromatic leukodystrophy. *N Engl J Med* 1991; **324**: 18.
18. Hohenschütz C, Eich P, Friedl W et al. Pseudodeficiency of arylsulfatase A: a common genetic polymorphism with possible disease implications. *Hum Genet* 1989; **82**: 45.
19. Baldinger S, Pierpont ME, Wenger DA. Pseudo deficiency of arylsulfatase A: a counseling dilemma. *Clin Genet* 1987; **31**: 70.
20. Fluharty AL. Arylsulfatase A deficiency. In: Pagon RA, Bird TD, Dolan CR, Stephens K (eds). *GeneReviews* [Internet]. Seattle, WA: University of Washington, 1993–2006, May 30.
21. Bonkowski JL, Nelson C, Kingston JL et al. The burden of inherited leukodystrophies in children. *Neurology* 2010; **24**: 718.
22. Kihara H. Metachromatic leukodystrophy, an unusual case with a subtle cerebroside sulfatase defect. In: Buchwald N, Brazier MAB (eds). *Brain Mechanisms in Mental Retardation*. New York: Academic Press, 1975: 501.
23. Zlotogora J, Costeff H, Elian E. Early motor development in metachromatic leukodystrophy. *J Neurol Neurosurg Psychiatry* 1973; **36**: 30.
24. Desilva KL, Pearce J. Neuropathy of metachromatic leukodystrophy. *J Neurol Neurosurg Psychiatry* 1973; **36**: 30.
25. Hagberg B. Clinical symptoms, signs and tests in metachromatic leukodystrophy. In: Folch-Pi J, Bauer H (eds). *Brain Lipids and Lipoproteins, and the Leukodystrophies*. Amsterdam: Elsevier, 1963: 134.
26. Cogan DG, Kuwabara T, Moser H. Metachromatic leukodystrophy. *Ophthalmologia* 1970; **160**: 2.
27. Morana G, Biancheri R, Dirocco M et al. Enhancing cranial nerves and cauda equina: an emerging magnetic resonance imaging pattern in metachromatic leukodystrophy and Krabbe disease. *Neuropediatrics* 2009; **40**: 291.
28. MacFaul R, Cavanagh N, Lake BD et al. Metachromatic leukodystrophy: review of 38 cases. *Arch Dis Child* 1982; **57**: 168.
29. Artigas O, Lagranha VL, Saraiva-Pereira ML et al. Clinical and biochemical study of 29 Brazilian patients with metachromatic leukodystrophy. *J Inher Metab Dis* 2010; July 2 [Epub ahead of print].
30. Balsev T, Cortez MA, Blaser SI, Haslam RHA. Recurrent seizures in metachromatic leukodystrophy. *Pediatr Neurol* 1997; **17**: 150.
31. Clarke JTR, Skomorowski MA, Chang PL. Marked clinical difference between two sibs affected with juvenile metachromatic leukodystrophy. *Am J Med Genet* 1989; **33**: 10.
32. Kappler J, Von Figura K, Gieselmann V. Late-onset metachromatic leukodystrophy: molecular pathology in two siblings. *Ann Neurol* 1992; **31**: 256.
33. Percy AK, Kaback MM, Herndon RM. Metachromatic leukodystrophy: comparison of early and late-onset forms. *Neurology* 1977; **27**: 933.
34. Manowitz P, Kling A, Kohn H. Clinical course of adult metachromatic leukodystrophy presenting as schizophrenia. *J Nerv Ment Dis* 1978; **166**: 500.
35. Alves D, Pires MM, Guimaraes A, Miranda MC. Four cases of late onset metachromatic leukodystrophy in a family: clinical, biochemical and neuropathological studies. *J Neurol Neurosurg Psychiatry* 1986; **49**: 1423.

36. Deeg KH, Reif R, Stehr K *et al.* Chronisch hämorrhagische Pankreatitis bei Gallenblasenpolypsis als Erstsymptom der metachromatischen Leukodystrophie. *Monatsschr Kinderheilkd* 1986; **134**: 272.
37. Tesluk H, Munn RJ, Schwartz MZ, Ruebner BH. Papillomatous transformation of the gallbladder in metachromatic leukodystrophy. *Pediatr Pathol* 1989; **9**: 741.
38. Siegel EG, Lucke H, Schauer W, Creutzfeldt W. Repeated upper gastrointestinal hemorrhage caused by metachromatic leukodystrophy of the gall bladder. *Digestion* 1992; **51**: 121.
39. Yavuz H, Yuksekkaya HA. Intestinal involvement in metachromatic leukodystrophy. *J Child Neurol* 2011; **26**: 117.
40. Duyff RF, Weinstein HC. Late-presenting metachromatic leukodystrophy. *Lancet* 1996; **348**: 1382.
41. Furst W, Sandhoff K. Activator proteins and topology of lysosomal sphingolipid catabolism. *Biochim Biophys Acta* 1992; **1126**: 1.
42. Brismar J. CT and MRI of the brain in inherited neurometabolic disorders. *J Child Neurol* 1992; **7**(Suppl.): S112.
43. Hyde TM, Ziegler JC, Weinberger DR. Psychiatric disturbances in metachromatic leukodystrophy. Insights into the neurobiology of psychosis. *Arch Neurol* 1992; **49**: 401.
44. Bosch EP, Hart MN. Late adult-onset metachromatic leukodystrophy: dementia and polyneuropathy in a 63-year-old man. *Arch Neurol* 1978; **35**: 475.
45. Fressinaud C, Vallat JM, Mason M *et al.* Adult-onset metachromatic leukodystrophy presenting as isolated peripheral neuropathy. *Neurology* 1992; **42**: 1396.
46. Kehrer C, Blumenstock G, Raabe C *et al.* Development and reliability of a classification system for gross motor function in children with metachromatic leukodystrophy. *Dev Med Child Neurol* 2011; **53**: 156.
47. Wenger DA, Degala G, Williams C *et al.* Clinical, pathological and biochemical studies on an infantile case of sulfatide/GM1 activator protein deficiency. *Am J Med Genet* 1989; **33**: 255.
48. Lu-Ning W, Ke-Wei H, Dong-Gang W, Ze-Yan L. Adult metachromatic leukodystrophy without deficiency of arylsulphatase. *Chinese Med J* 1990; **103**: 846.
49. Fukumizu M, Matsui K, Hanaoka S *et al.* Partial seizures in two cases of metachromatic leukodystrophy: electrophysiologic and neuroradiologic findings. *J Child Neurol* 1992; **7**: 381.
50. Blom S, Hagberg B. EEG findings in late infantile metachromatic and globoid cell leukodystrophy. *Electroencephalogr Clin Neurophysiol* 1967; **22**: 253.
51. Klemm E, Conzelmann E. Adult-onset meta-chromatic leukodystrophy presenting without psychiatric symptoms. *J Neurol* 1989; **236**: 427.
52. Clark JR, Miller RG, Vidgoff JM. Juvenile-onset metachromatic leukodystrophy: biochemical and electrophysiologic studies. *Neurology* 1979; **29**: 346.
53. Pilz H, Hopf HC. A preclinical case of late adult metachromatic leukodystrophy: neurophysiological findings. *J Neurol Neurosurg Psychiatry* 1972; **35**: 360.
54. Wulff CH, Trujaborg W. Adult metachromatic leukodystrophy: neurophysiologic findings. *Neurology* 1985; **35**: 1776.
55. Cruz AM, Ferrer MT, Fueyo E, Galadós L. Peripheral neuropathy detected on an electrophysiological study as the manifestation of MLD in infancy. *J Neurol Neurosurg Psychiatry* 1975; **38**: 169.
56. Brown FR, Shimizu H, McDonald JM *et al.* Auditory evoked brainstem response and high-performance liquid chromatography sulfatase assay as early indices of metachromatic leukodystrophy. *Neurology* 1981; **31**: 980.
57. Suárez EC, Rodríguez AS, Tapia AG *et al.* Ichthyosis: the skin manifestation of multiple sulfatase deficiency. *Pediatr Dermatol* 1997; **14**: 369.
58. Jayakumar PN, Aroor SR, Jha RK, Arya BYT. Computed tomography (CT) in late infantile metachromatic leukodystrophy. *Acta Neurol Scand* 1989; **79**: 23.
59. Kim TS, Kim IO, Kim WS *et al.* MR of childhood metachromatic leukodystrophy. *Am J Neuroradiol* 1997; **18**: 733.
60. Dali C, Hanson LG, Barton NW *et al.* Brain N-acetylaspartate levels correlate with motor function in metachromatic leukodystrophy. *Neurology* 2010; **75**: 1896.
61. Chang XZ, Liu JY, Wu Y *et al.* Detection of vacuolated peripheral blood lymphocytes in screening for and diagnosis of lysosomal storage diseases. *Zhonghua Er Ke Za Zhi* 2011; **49**: 135.
62. Fuller M, Tucker JN, Lang DL *et al.* Screening patients referred to a metabolic clinic for lysosomal storage disorders. *J Med Genet* 2011; **48**: 422.
63. Gustavson K-H, Hagberg B. The incidence and genetics of metachromatic leukodystrophy in northern Sweden. *Acta Paediatr Scand* 1971; **60**: 585.
64. Zlotogora J, Bach G, Barak V, Elian E. Metachromatic leukodystrophy in the Habbanite Jews: high frequency in a genetic isolate and screening for heterozygotes. *Am J Hum Genet* 1980; **32**: 663.
65. Fujii T, Kobayashi T, Honke K *et al.* Proteolytic processing of human lysosomal arylsulfatase A. *Biophys Acta* 1992; **1122**: 93.
66. Tonnesen T, Bro PV, Brondum Nielsen K, Lykkelund C. Metachromatic leukodystrophy and pseudoaryl-sulfatase A deficiency in a Danish family. *Acta Paediatr Scand* 1983; **72**: 175.
67. Thomas GH, Howell RR. Arylsulfatase A activity in human urine: quantitative studies on patients with lysosomal disorders including metachromatic leukodystrophy. *Clin Chim Acta* 1972; **36**: 99.
68. Percy AK, Brady RO. Metachromatic leukodystrophy: diagnosis with samples of venous blood. *Science* 1968; **161**: 594.
69. Porter MT, Fluharty AL, Kihara H. Metachromatic leukodystrophy: arylsulfatase-A deficiency in skin fibroblast cultures. *Proc Natl Acad Sci USA* 1978; **62**: 887.
70. Kaback MM, Howell RR. Infantile metachromatic leukodystrophy: heterozygote detection in skin fibroblasts and possible applications to intrauterine diagnosis. *N Engl J Med* 1970; **282**: 1336.
71. Kappler J, Leinekugel P, Conzelmann E *et al.* Genotype-phenotype relationship in various degrees of arylsulfatase A deficiency. *Hum Genet* 1991; **86**: 463.
72. Tamaka A, Higami S, Isshiki G *et al.* Immunofluorescence staining, and immunological studies of arylsulfatase deficiency (MSD) and metachromatic leukodystrophy (MLD) fibroblasts. *J Inherit Metab Dis* 1983; **6**: 21.

73. Porter MT, Fluharty A, Trammell J, Kihara H. A correlation of intracellular cerebroside sulfatase activity in fibroblasts with latency in metachromatic leukodystrophy. *Biochem Biophys Res Commun* 1971; **44**: 660.
74. Inui K, Emmett M, Wenger DA. Immunological evidence for deficiency in an activator protein for sulfatide sulfatase in a variant form of metachromatic leukodystrophy. *Proc Natl Acad Sci USA* 1983; **80**: 3074.
75. DuBois G, Turpin JC, Baumann N. Absence of ASA activity in healthy father of patient with metachromatic leukodystrophy. *N Engl J Med* 1975; **293**: 302.
76. Lott IT, Dulalney JT, Milunsky A *et al*. Apparent biochemical homozygosity in two obligatory heterozygotes for metachromatic leukodystrophy. *J Pediatr* 1976; **89**: 438.
77. DuBois G, Harzer K, Baumann N. Very low arylsulfatase A and cerebroside sulfatase activities in leukocytes of healthy members of metachromatic leukodystrophy family. *Am J Hum Genet* 1977; **29**: 191.
78. Hors-Cayla MC, Heuertz S, Van Cong N *et al*. Confirmation of the assignment of the gene for arylsulfatase A to chromosome 22 using somatic cell hybrids. *Hum Genet* 1979; **49**: 33.
79. Gustavson K-H, Arancibia W, Eriksson U, Svennerholm L. Deleted ring chromosome 22 in a mentally retarded boy. *Clin Genet* 1986; **29**: 337.
80. Phelan MC, Thomas GR, Saul RA *et al*. Cytogenetic, biochemical and molecular analyses of a 22q13 deletion. *Am J Med Genet* 1992; **43**: 872.
81. Inui K, Kao F-T, Fujibayashi S *et al*. The gene coding for a sphingolipid activator protein, SAP-1, is on human chromosome 10. *Somat Cell Mol Genet* 1985; **69**: 197.
82. Kao F-T, Law ML, Hartz J *et al*. Regional localization of the gene coding for sphingolipid activator protein SAP-1 on human chromosome 10. *Somat Cell Mol Genet* 1987; **13**: 685.
83. Kreysing J, Von Figura K, Gieselmann V. Structure of the arylsulfatase A gene. *Eur J Biochem* 1990; **191**: 627.
84. Berger J, Loschl B, Bernheimer H *et al*. Occurrence, distribution, and phenotype of arylsulfatase A mutations in patients with metachromatic leukodystrophy. *Am J Med Genet* 1997; **69**: 335.
85. Gieselmann V, Polten A, Kreysing J *et al*. Molecular genetics of metachromatic leukodystrophy. *Dev Neurosci* 1991; **13**: 222.
86. Fluharty AL, Fluharty CB, Bohne WI. Two new arylsulfatase A (ARSA) mutations in a juvenile metachromatic leukodystrophy (MLD) patient. *Am J Hum Genet* 1991; **49**: 1340.
87. Bohne W, Von Figura K, Gieselmann V. An 11-bp deletion in the arylsulfatase A gene of a patient with late infantile metachromatic leukodystrophy. *Hum Genet* 1991; **49**: 1340.
88. Kondo R, Wakamatsu N, Yoshino H *et al*. Identification of a mutation in the arylsulfatase A gene of a patient with adult-type leukodystrophy and Gaucher disease. *Am J Hum Genet* 1991; **48**: 971.
89. Eto Y, Kawame H, Hasegawa Y *et al*. Molecular characteristics in Japanese patients with lipidosis: novel mutations in metachromatic leukodystrophy and Gaucher disease. *Mol Cell Biochem* 1993; **119**: 179.
90. Barth ML, Fensom A, Harris A. Prevalence of common mutations in the arylsulphatase A gene in metachromatic leukodystrophy patients diagnosed in Britain. *Hum Genet* 1993; **91**: 73.
91. Al-Hassnan ZN, Al Dhalaan H, Patay Z *et al*. Sphingolipid activator protein B deficiency: report of 9 Saudi patients and review of the literature. *J Child Neurol* 2009; **24**: 1513.
92. Hayashi T, Nakamura M, Ichiba M *et al*. Adult-type metachromatic leukodystrophy with compound heterozygous ARSA mutations: a case report and phenotypic comparison with a previously reported case. *Psychiatry Clin Neurosci* 2011; **65**: 105.
93. Shukla P, Vasisht S, Srivastava R *et al*. Molecular and structural analysis of metachromatic leukodystrophy patients in Indian population. *J Neurol Sci* 2011; **301**: 38.
94. Kang DH, Lee DH, Hong YH *et al*. Identification of a novel splicing mutation in the ARSA gene in a patient with late-infantile form of metachromatic leukodystrophy. *Korean J Lab Med* 2010; **30**: 516.
95. Kihara H, Meek WE, Fluharty AL. Attenuated activities and structural alterations of arylsulfatase A in tissues from subjects with pseudo arylsulfatase A deficiency. *Hum Genet* 1986; **74**: 59.
96. von Figura K, Steckel F, Hasilik A. Juvenile and adult metachromatic leukodystrophy: partial restoration of arylsulfatase A (cerebroside sulfatase) activity in inhibitors of thiol proteinases. *Proc Natl Acad Sci USA* 1983; **80**: 6066.
97. Gieselmann V, Zlotogora J, Harris A *et al*. Molecular genetics of metachromatic leukodystrophy. *Hum Mutat* 1994; **4**: 233.
98. Porter MT, Fluharty AL, Harris SE, Kihara H. The accumulation of cerebroside sulfates by fibroblasts in culture from patients with late infantile metachromatic leukodystrophy. *Arch Biochem Biophys* 1970; **138**: 646.
99. O'Brien JS, Kishimoto Y. Saposin proteins: structure, function and role in human lysosomal storage disorders. *FASEB J* 1991; **5**: 301.
100. Kretz KA, Carson GS, Morimoto S *et al*. Characterization of a mutation in a family with saposin B deficiency: a glycosylation site defect. *Proc Natl Acad Sci USA* 1990; **87**: 2541.
101. Raghavan SS, Gajewski A, Kolodny EH. Leukocyte sulfatidase for the reliable diagnosis of metachromatic leukodystrophy. *J Neurochem* 1981; **36**: 724.
102. Eto Y, Tahara T, Koda N *et al*. Prenatal diagnosis of metachromatic leukodystrophy. A diagnosis by amniotic fluid and its confirmation. *Arch Neurol* 1982; **39**: 29.
103. Percy AK, Farrell DF, Kaback MM. Cerebroside sulphate (sulphatide) sulphohydrolase: an improved assay method. *J Neurochem* 1972; **19**: 233.
104. Svennerholm L. Some aspects of the biochemical changes in leukodystrophy. In: Folch-Pi J, Bauer H (eds). *Brain Lipids and Lipoproteins, and the Leucodystrophies*. Amsterdam: Elsevier, 1963: 104.
105. Blomqvist M, Gieselmann V, Mansson JE. Accumulation of lysosulfatide in the brain of arylsulfatase A-deficient mice. *Lipids Health Dis* 2011; **10**: 28.
106. Pilz H, Muller D, Linke L. Histochemical and biochemical studies of urinary lipids in metachromatic leukodystrophy and Fabry disease. *J Lab Clin Med* 1973; **81**: 7.
107. Philippart M, Sarlieve L, Meurant C, Mechler L. Human urinary sulfatides in patients with sulfatidosis (metachromatic leukodystrophy). *J Lipid Res* 1971; **12**: 434.

108. Natowicz MR, Prenc EM, Chaturvedi P, Newburg DS. Urine sulfatides and the diagnosis of metachromatic leukodystrophy. *Clin Chem* 1996; **42**: 232.
109. Lugowska A, Tytki-Symanska A, Berger J, Molzer B. Elevated sulfatide excretion in compound heterozygotes of metachromatic leukodystrophy and ASA-pseudodeficiency allele. *Clin Biochem* 1997; **30**: 325.
110. Fluharty AL. Arylsulfatase A deficiency. In: Pagon RA, Bird TD, Dolan CR, Stephens K (eds). GeneReviews [Internet]. Seattle, WA: University of Washington, 1993–2006, May 30.
111. Jaeken J, Casaer P, De Cock P, Francois B. Vigabatrin in GABA metabolism disorders. *Lancet* 1989; **1**: 1074.
112. Krivit W, Shapiro E, Kennedy W *et al*. Treatment of late infantile metachromatic leukodystrophy by bone marrow transplantation. *N Engl J Med* 1990; **322**: 28.
113. Shapiro E, Lipton ME, Krivit W. White matter dysfunction and its neuropsychological correlates: a longitudinal study of a case of metachromatic leukodystrophy treated with bone marrow transplant. *J Clin Exp Neuropsychiatry* 1992; **14**: 610.
114. Pridjian G, Humbert J, Willis J, Shapiro E. Presymptomatic late-infantile metachromatic leukodystrophy treated with bone marrow transplantation. *J Pediatr* 1994; **125**: 755.
115. Krivit W, Shapiro EG. Bone marrow transplantation for storage diseases. In: Desnick RJ (ed.). *Treatment of Genetic Disease*. New York: Churchill Livingstone, 1991: 203.
116. Krivit W, Lockman LA, Watkins PA *et al*. The future for treatment by bone marrow transplantation for adrenoleukodystrophy, metachromatic leukodystrophy, globoid cell leukodystrophy and Hurler syndrome. *J Inherit Metab Dis* 1995; **18**: 398.
117. Shapiro EG, Lockman LA, Balthazor M, Krivit W. Neuropsychological outcomes of several storage diseases with and without bone marrow transplantation. *J Inherit Metab Dis* 1995; **18**: 413.
118. Miyake N, Miyake K, Karlsson S, Shimada T. Successful treatment of metachromatic leukodystrophy using bone marrow transplantation of HoxB4 overexpressing cells. *Mol Ther* 2010; **18**: 1373.
119. Smith NJ, Marcus RE, Sahakian BJ *et al*. Haematopoietic stem cell transplantation does not retard disease progression in the psycho-cognitive variant of late-onset metachromatic leukodystrophy. *J Inherit Metab Dis* 2010, Nov 16 [Epub ahead of print].
120. Cable C, Finkel RS, Lehky TJ *et al*. Unrelated umbilical cord blood transplant for juvenile metachromatic leukodystrophy: a 5-year follow-up in three affected siblings. *Mol Genet Metab* 2011; **102**: 207.
121. Matthes F, Wolte P, Bockenhoff A *et al*. Transport of arylsulfatase A across the blood–brain barrier *in vitro*. *J Biol Chem* 2011; **286**: 17487.

Multiple sulfatase deficiency

Introduction	769	Treatment	776
Clinical abnormalities	770	References	777
Genetics and pathogenesis	775		

MAJOR PHENOTYPIC EXPRESSION

Facial and somatic features and dysostosis multiplex of a mucopolysaccharidosis; ichthyosis; neurologic features of a late infantile metachromatic leukodystrophy; mucopolysacchariduria; defective activity of arylsulfatase A, B, and C, steroid sulfatase and the mucopolysaccharide sulfatases, including iduronate sulfatase, heparan-N-sulfatase, N-acetylgalactosamine-6-sulfatase, and N-acetylglucosamine-6-sulfatase; and defective post-translational change of sulfatase cysteine-69 to aminopropionic acid.

INTRODUCTION

Multiple sulfatase deficiency (MSD) was reported in 1965 by Austin, Armstrong and Shearer [1, 2] as a metachromatic leukodystrophy (MLD) in which there were also features of mucopolysaccharidosis. Deficient activity of a number of sulfatases led to the designation of multiple sulfatase deficiency [3]. At least seven enzymes are now known to be deficient [2–5]. The fundamental defect [6–8] represents a novel mechanism of disease in which the mutation is in an enzyme responsible for post-translational change of a cysteine moiety of each of the sulfatases, a change that conveys activation of the enzyme (Figure 102.1). This cysteine is conserved in each of the sulfatases (Table

Table 102.1 Homology of sulfatase sequences

Arylsulfatase A	L	C	T	P	S	R
Arylsulfatase B	L	C	T	P	S	R
Steroid sulfatase	L	C	T	P	S	R
N-Acetylglucosamine-6-sulfatase	L	C	C	P	S	R
Iduronate sulfatase	V	C	A	P	S	R
N-Acetylgalactosamine-6-sulfatase	L	C	S	P	S	R
Sea urchin arylsulfatase	V	C	T	P	S	R

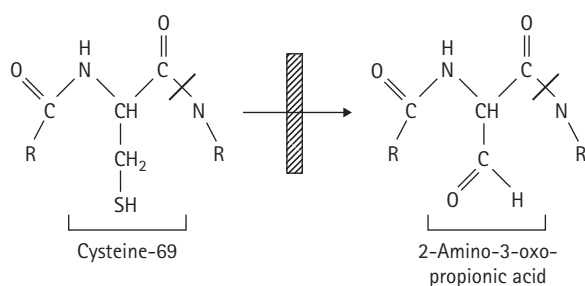


Figure 102.1 Mechanism of activation of sulfatase enzymes by conversion of a highly conserved cysteine at position 69 in arylsulfatase A to 2-amino-3-oxopropionic acid.

102.1). It is converted to 2-amino-3-oxopropionic acid (formylglycine) (Figure 102.1).

This post-translational change affects the entire sulfatase family, at least seven members of which are lysosomal enzymes that are specifically involved in the degradation of sulfated glycosaminoglycans, sulfolipids, or other sulfated molecules. It leads to combined deficiencies of all of the sulfatases.

The enzyme, formylglycine-generating enzyme (FGE), catalyzes the oxidation of the cysteine residue at position 64 to 2-amino-3-oxopropionic acid. This is the amino acid which enables the sulfate ester hydrolysis. It is located in the endoplasmic reticulum [8].

The gene has been identified, and missense mutations have been discovered which lead to variable loss of function [9]. The gene sulfatase modifying factor 1

(*SUMF1*) encodes the enzyme, which normally activates sulfatases by modifying this key cysteine residue within the catalytic domains of each sulfatase. In patients with multiple sulfatases, the mutated enzyme is retained in the endoplasmic reticulum (ER). This is the site of its enzymatic action on nascent sulfatases. *SUMF1* interacts with protein disulfide isomerase (PDI) and ERp44, two thioredoxin family members of the early secretory pathway, and with ERGIC-53, a lectin that shuttles between the ER and the Golgi. Functional assays reveal that these interactions are crucial for controlling *SUMF1* traffic and function [10].

Deficiency of arylsulfatase A would be consistent with clinical features of MLD. The deficiency of steroid sulfatase would be responsible for the skin lesions of X-linked ichthyosis. Among the enzymes of mucopolysaccharide metabolism: (1) deficiency of iduronate sulfatase would give manifestations of Hunter syndrome; (2) deficiency of heparan sulfatase could yield the impaired mental development and cerebral features of Sanfilippo A disease; (3) deficiency of N-acetylglucosamine-6-sulfatase, those of Sanfilippo B disease; (4) deficiency of N-acetylgalactosamine-6-sulfatase would give rise to features of Morquio disease; and (5) deficiency of N-acetylgalactosamine-4-sulfatase, also known as arylsulfatase B, would cause features of Maroteaux-Lamy disease, including corneal clouding. Obviously, different degrees of deficiency or amounts of residual enzyme activity would be expected to lead to quite different phenotypes. A number of different clinical phenotypes have been delineated [4], including the classic late infantile form, a neonatal form, a juvenile form, and a Saudi variant.

CLINICAL ABNORMALITIES

The clinical phenotype of MSD represents a summation of the various enzymatic defects [4, 5, 7, 11] a combination of the clinical features found in diseases resulting from deficiency of the individual sulfatases: mucopolysaccharidoses II, IIIA, IIID, IVA, and VI, metachromatic leukodystrophy, X-linked ichthyosis, and the X-linked recessive form of chondrodysplasia punctata [12]. The phenotypic outcome in MSD depends on the degree of residual FGE enzyme activity and on protein stability.

In its classic form, the disease presents in late infancy with the symptoms of the progressive degeneration of myelin of MLD [11], or in the neonatal form with a picture of a severe mucopolysaccharidosis [12]. A milder juvenile form with onset at about five years of age has been reported [6].

The more commonly described presentation is that of an MLD [5, 7, 11] with mild features of mucopolysaccharidosis [13, 14]. Early development may be normal, and patients may walk and speak at normal times [4], but some may be developmentally delayed early on. During the second or third year, milestones attained are slowly lost.

Increased deep tendon reflexes and ankle clonus may be followed by spastic quadripareisis. There may be seizures. Neurodegeneration is progressive to blindness and loss of hearing [11, 15], and deafness may be severe. The head develops microcephaly [9, 16, 17]. Swallowing becomes



Figure 102.2 Multiple sulfatase deficiency (MSD). A patient with MSD whose eyes were strikingly proptotic. He also had atlantoaxial dislocation. Sulfatase activities were very low.



Figure 102.3 IQ: This girl with multiple sulfatase deficiency also had strikingly prominent eyes.



Figure 102.4 MM: A boy with multiple sulfatase deficiency. Facial features were coarse, the nasal bridge depressed, and the nasal tip tilted, highlighting the abundant nasal subcutaneous tissue. The skin of the legs, as well as the torso illustrated, was ichthyotic.



Figure 102.6 HZ: The abdomen was protruberant as a consequence of hepatosplenomegaly.



Figure 102.5 HZ: A girl with multiple sulfatase deficiency. Hirsutism was very pronounced and the facial features coarse.



Figure 102.7 MM: The hands were just like those of a patient with Hurler disease.

difficult, and tube feeding is required. Death may occur at 10–18 years, but there has been survival into the third decade.

Mucopolysaccharidosis-like features maybe evident

very early in life [12, 18] and may well be the first evidence of disease. The diagnosis should be considered in young patients with signs of mucopolysaccharidosis. The facial features are coarse, and there is hirsutism (Figures 102.2, 102.3, 102.4, and 102.5). There may be stertorous breathing, nasal discharge, or hernias. Hepatosplenomegaly may be prominent (Figure 102.6). Joints become stiff and there may be contractures. The claw hand may be identical to that of Hurler disease (Figures 102.7 and 102.8). Virtually all patients have roentgenographic evidence of dysostosis multiplex (Chapters 76 and 77). The initial diagnosis may be of Hunter or Sanfilippo disease [5, 18]. Cardiac complications have been observed [12]. A few patients do not appear to have recognizable clinical features



Figure 102.8 AA: Another patient with multiple sulfatase deficiency and very striking hands. A brother also had MSD.



Figure 102.9 AA: The gingival hyperplasia was as striking as that seen in I-cell disease (Chapter 83). The teeth were carious.

of mucopolysaccharidosis. Ophthalmologic findings have included optic atrophy, retinal degeneration, and nystagmus [5, 10, 19]. The cornea is usually clear [5, 9, 18]. Two patients have had a cherry red macula [13, 20]. The classic presentation is with ichthyosis (Figure 102.4) [5, 16, 17].

Another neonatal phenotype has been distinguished [4] in which there is presentation at birth with prominent features of mucopolysaccharidosis, severe encephalopathy, and early demise [12, 15, 21]. Hepatosplenomegaly is pronounced. These patients have also had ichthyosis by two to three years of age [12, 21]. The neck is short, and there is hypoplasia of the vertebral bodies and epiphyseal dysplasia. Consistent with the severity of the phenotype, enzyme activities of all the sulfatases tested were very severely depressed [12, 21].

A Saudi variant has been distinguished [4] in which there was early infantile onset of severe dysostosis multiplex, appearing as Maroteaux-Lamy syndrome or Morquio disease. There was corneal clouding in six of eight patients. Facial features were coarse. The orbit may appear shallow



Figure 102.10 AR: A patient with multiple sulfatase deficiency had an unusually shaped head and a prominent keel or frontal bridge.



Figure 102.11 MM: A patient with multiple sulfatase deficiency who was macrocephalic. Facial features were coarse and he was hirsute.

and the eyes proptotic (Figures 102.2, 102.3, and 102.15). Deafness was absent, but one patient had abnormal auditory evoked potentials on one side. Ichthyosis was absent, and in six of seven patients studied the activity of steroid sulfatase was normal [4]. On the other hand, we



Figure 102.12 Stature was short and the neck particularly short. Flexion of the hips and knees, as well as the elbows contributed to the Morquio-like appearance.



Figure 102.13 AQ: A patient who also had Morquio-like habitus.

have since seen ichthyosis in another Saudi patient. Patients had mild to moderately impaired mental development, and one patient had a normal cognitive quotient despite motor impairment. There may be macrocephaly (Figures 102.9 and 102.10) and gingival hyperplasia (Figure 102.11).



Figure 102.14 Seven-year-old boy with Morquio-like stature and coarse face, typical of Austin's disease.

Most of these patients had retinal changes, but two had lenticular opacities, which have been seen, but rarely in the classic presentation [5]. Stature was short (Figures 102.12 and 102.13), often with a crouching, Morquio-like position because of contractures. Two patients had evidence of cervical cord compression with the development of sudden quadriparesis, followed in one by death.

A juvenile-onset form of MSD was reported by Tanaka *et al.* [22] in which there was onset at five years of a slowly progressive quadriplegia, retinitis, and blindness. There was ataxia and dysarthria. Hepatomegaly was only moderate. Stature was short and the skin was ichthyotic. Death was at 26 years.

Roentgenograms usually reveal some degree of dysostosis multiplex (Figures 102.14 and 102.15). In the Saudi patients, premature synostosis of one or more cranial sutures led to deformities, such as trigonocephaly, brachycephaly, or dolichocephaly [4]. Macrocephaly was seen, as well as the microcephaly more common in other forms of MSD. Some patients have had J-shaped sellas. Abnormalities of the odontoid have been observed [4]; C1 has been lower than normal. The posterior arch has been anterior, compressing the cord. There has been anterior subluxation of the atlas and hypoplasia of C2. Nerve conduction may be slowed.

Neuroimaging of the brain by computed tomography (CT) or magnetic resonance imaging (MRI) (Figures 102.16, 102.17, 102.18, 102.19, and 102.20) reveals a symmetric decrease in attenuation of white matter, with high T₂ signal throughout the white matter. White matter changes were seen in all the Saudi patients [4]. One had

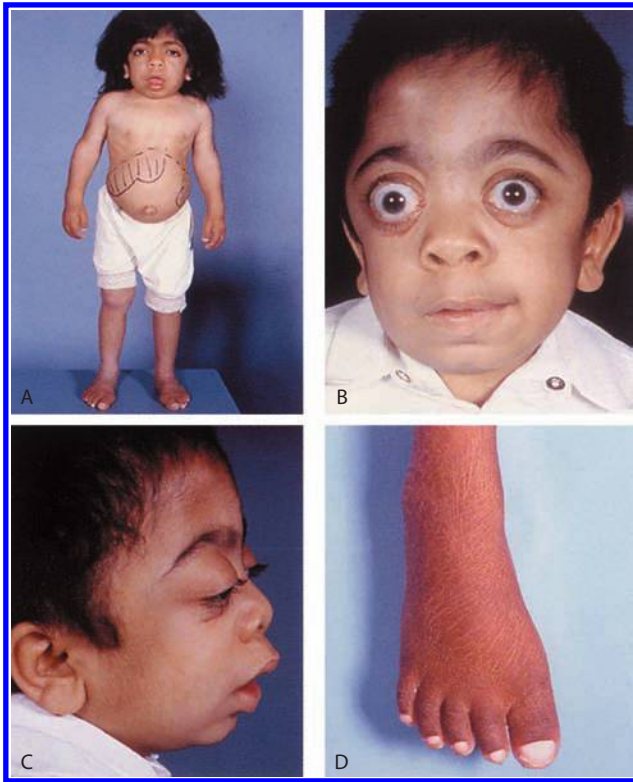


Figure 102.15 Phenotypic features of Austin disease. Typical stature (Morquio-like) (A). Typical face with coarse features (B and C), and ichthyosis (D). (B) is the same as Figure 102.2.

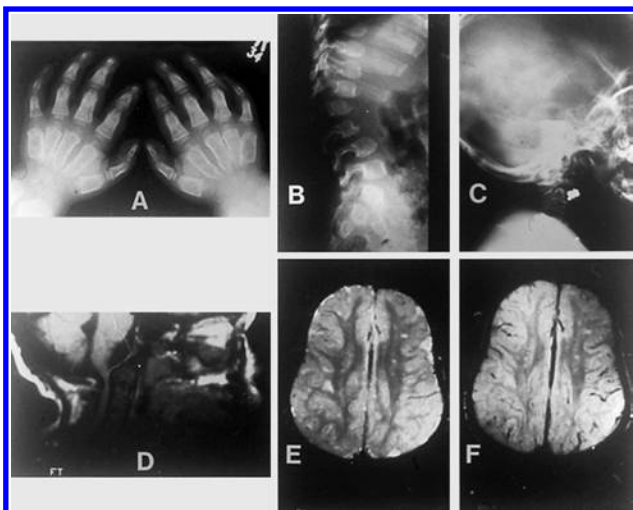


Figure 102.16 Radiological features of Austin disease, with typical hands (A), gibbus deformity (B), J-shaped sella (C), odontoid hypoplasia with C1-C2 posterior dislocation (D), and leukodystrophy (E and F).

hydrocephalus and one an arachnoid cyst. MRI in a nine-month-old child with MSD demonstrated extensive diffuse symmetrical high signal in the deep white matter of both cerebral hemispheres, as well as of the subcortical white matter and the brainstem, while there was additional



Figure 102.17 The hands of RI (Figure 102.12) illustrated the characteristic broad, short proximally tapering metacarpals and broad phalanges.



Figure 102.18 Roentgenogram of the spine of RI, illustrating advanced dysostosis multiplex. The ribs were broad and spatulate. The vertebrae were ovoid and the lumbar vertebrae had anterior hooks.

enlargement of sulci and subdural spaces and mild atrophy [23].

Laboratory findings in all these patients include mucopolysacchariduria (dermatan sulfate and heparan sulfate). Alder-Reilly granules are found in leukocytes of the bone marrow and peripheral blood. The cerebrospinal

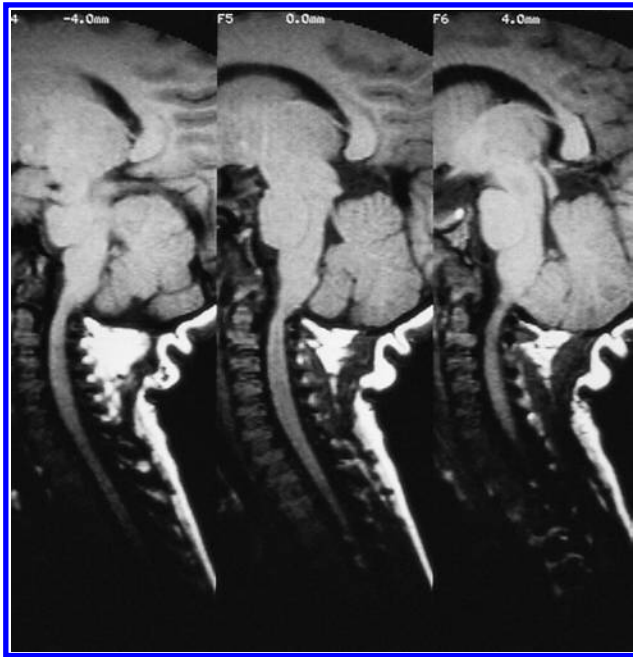


Figure 102.19 Magnetic resonance image of RF, a patient with multiple sulfatase deficiency. A localized quite marked constriction of the anteroposterior diameter and compression of the cord to about one-half of normal size.

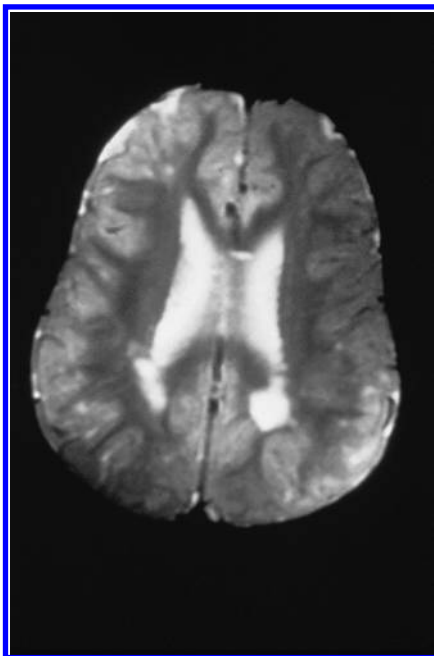


Figure 102.20 Magnetic resonance image of the brain of SR, a four-year-old patient with multiple sulfatase deficiency. Moderately extensive high T_2 changes in the subcortical white matter were present bilaterally.

fluid (CSF) protein concentration may be elevated. Diagnosis is made by confirming the deficiency in the activity of a number of sulfatase enzymes [12].

Newborn screening for lysosomal storage diseases

has been developed on the basis of quantification of immunoreactive lysosomal proteins [24]. A multiplex assay for 14 enzyme proteins studied 1415 dried blood spots and identified a patient with multiple sulfatase deficiency on the basis of reduced amounts of many sulfatase proteins. In this study, sensitivity and specificity was good; there was one false negative, a patient with mucopolysaccharidosis II [25].

GENETICS AND PATHOGENESIS

Transmission is autosomal recessive. Both sexes have been equally represented, often with more than one affected patient in a sibship. Consanguinity has been documented. Prevalence in Australia has been reported as one in 1.4 million [26].

The locus has been shown by complementation studies in somatic cell hybrids to be different from that of MLD. Fusion of MLD and MSD fibroblasts led to correction of arylsulfatase A deficiency [27]. Hybridization of cells of the classic and neonatal forms of MSD was not complementary, and enzyme activity was not restored [28].

Defective activity of sulfatases can be shown in cultured fibroblasts [18, 19, 29] and in tissues, such as kidney, brain, and liver [2, 12, 29, 30]. There has been some correlation of the levels of residual enzyme activity and clinical phenotype [3, 28, 31, 32]. In general, classic patients have had severe deficiency [3, 33, 34], while activity is absent or certainly less than 10 percent of control in the neonatal patients [21, 33]. In the Saudi patients, activities ranged from 3–10 percent of arylsulfatase A to 20–41 percent of control of the Sanfilippo A enzyme [4].

In the juvenile patients of Tanaka *et al.* [22], lymphocytes and fibroblasts displayed 20–60 percent of control levels. Cultured fibroblasts of the neonatal patients incorporated significantly greater amounts of ^{35}S -labeled sodium sulfate into acid mucopolysaccharide than did those of cells of classic MSD patients [35]. Prenatal diagnosis has been reported [36] by sulfatase assay of cultured amniocytes or chorionic villus cells. In one patient with MSD, a low estriol was recorded in maternal urine [37], and in another with neonatal MSD, there was placental hormone deficiency [21]. Heterozygotes have been reported [38] to have intermediate activities of sulfatase in cultured fibroblasts.

As in MLD, urinary excretion of sulfatide is elevated. Sulfatide levels in the CSF are also increased. Accumulation of cholesteryl sulfate has been identified in liver, kidney, plasma, and urine. Cerebral gangliosides have been abnormal, as in Hunter disease [10, 18, 21].

The activity of sulfatases in cultured fibroblasts can be influenced by additions to the medium. Activity may be increased by the substitution of N-2-hydroxymethylpiperazine-N-2-sulfonic acid for bicarbonate buffer [34], and addition of leupeptin, a thiol protease inhibitor, leads to the appearance of arylsulfatase A activity and the ability to degrade labeled sulfatides

[39]. These observations are consistent with the concept that the synthesis of the enzymes may be normal but their degradation is rapid [39]. Furthermore, isolated individual sulfatases from MSD material had normal kinetic properties [29, 34]. It was postulated that the mutation is an enzyme responsible for co- or post-translational modification of the sulfatase polypeptides [40]. Relevant to this hypothesis are the results of gene transfer of the cDNA for the arylsulfatases into fibroblasts [41]: cDNAs from MSD sources were expressed in normal and MSD cells. In MSD cells, mature enzyme proteins were present, but they were less than 5 percent as active as normal. This would fit with failure of an enzyme responsible for activation through modification.

In an elegant series of experiments, Schmidt *et al.* [6] showed that a cysteine predicted from the cDNA sequence that is conserved among all known sulfatases is replaced by a 2-amino-3-oxopropionic acid in active enzymes, while in sulfatases of MSD cells, the cysteine is unchanged (Figure 102.1). In arylsulfatase A, the cysteine of position 69 was found to be replaced with a compound containing no sulfur and an aldehyde function on tandem mass spectrometry, and the compound was definitely identified as 2-amino-3-oxopropionic acid in both arylsulfatase A and B. In MSD fibroblasts, the residue was cysteine. These observations have uncovered a novel modification of enzyme that confers catalytic activity on the protein. It is clearly the enzyme that catalyzes this modification that is defective in MSD.

As in the case of patients with multiple sulfatase deficiency *Sumf1*($-/-$) mice die early, and they have congenital growth, impairment and skeletal and neurological abnormalities. Vacuoles are visualized in histologic specimens, and there is significant storage of glycosaminoglycans in lysosomes. Macrophages were the predominant site of vacuolar lysosomal storage [42]. The gene *SUMF1* was cloned independently by Cosma *et al.* [43] and Dierks *et al.* [44] and mapped to chromosome 3p26 [44]. It contains nine exons spanning 105 kb. Among mutations identified, a 4 bp deletion (GTAA) at position 5 of intron 3 leads to loss of the splice donor site for the intron and in-frame deletion of exon 3. It has been referred to as IVS 3 + 5-8 del [44] and 519 + 4 del GTAA [43]. One patient had a C to T transversion on the other allele at nucleotide 1076 (S359X) and another had R327Y on the other allele [43, 44]. In addition to a 1 bp deletion at 276C, missense mutations identified were: R345C, C218Y, A348P, and M1V [43]. Expression of the *SUMF1* genes carrying missense mutations revealed loss of function [9]. A compound heterozygote for two novel mutations (p.R349G and p.F244S) was reported in Brazilian patients [12]. A novel missense mutation c.739G > C causing a p.G247R amino acid substitution in the *SUMF1* protein was reported in a Turkish family [45]. Four missense mutations p.A177P, p.W179S, p.A279V, and p.R349W did not affect localization of the FGE enzyme in the endoplasmic reticulum of MSD fibroblasts. The mutations did decrease the specific activity of the enzyme to less than 1 percent (p.A177P and p.R349W), 3 percent

(p.W179S), or 23 percent (p.A279V). Protein stability was severely impaired in p.A279V and p.R349W, and almost comparable to wild type in p.A177P and p.W179S. The patient with the mildest clinical phenotype carried the mutation p.A279V leading to decreased FGE protein stability, but high residual enzymatic activity and only slightly reduced sulfatase activity. In contrast, the most severely affected patient carried the p.R349W mutation with drastically reduced protein stability, and residual enzymatic activity activities [46].

Remodeled heparan sulfate proteoglycans (HSPG) are involved in the differentiation of hematopoietic stem cells. *SUMF1* activates both Sulf1 and Sulf2 sulfatases, which act to remodel heparan sulfate proteoglycans. The effects of these mutations were reversed by blockade of the fibroblast growth factor (FGF) pathway in *Sumf1*($-/-$) mice [47].

Evidence of a generalized abnormality of T-cell development was found in multiple sulfatase-deficient mice [48]. In the knockout mouse model of MSD, there were enhanced apoptotic markers. Numbers of autophagosomes were increased compared to wild-type mice. This was thought to result from impaired autophagosome-lysosome fusion. The conceptualization of disorders of autophagy would fit with the conceptual idea of lysosomal diseases as neurodegenerative [49]. Autophagy is involved in recycling of proteins and organelles that follows fusion of autophagosomes with lysosomes. Lysosomal dysfunction impairs autophagy. Inactivation of *Sumf1* and the accumulation of glycosaminoglycans in lysosomes led to disturbed autophagy [50].

TREATMENT

The symptomatic treatment of the leukodystrophy is supportive. Nasogastric or gastrostomy feeding may be required. Surgical fusion to stabilize the upper cervical spine may save the life of a patient or avert disabling quadriplegia. Magnetic resonance imaging is helpful in identifying candidates for surgery. Bone marrow transplantation has met with limited success in MLD and in various mucopolysaccharidoses. Experience is not available in MSD. Enzyme replacement therapy in mucopolysaccharidosis has encouraged the development of other enzymes for this purpose, including the sulfatases. The development of the expressed product of the *SUMF1* gene has potential for the treatment of individual sulfatase deficiencies, as well as of MSD [9].

The discovery of FGE as the sulfatase-activating enzyme has made more logical the possibilities of enzyme replacement or gene therapy [8]. Approaches to gene therapy have been explored in the *Sumf1* knockout mouse model (*Sumf1*($-/-$)), in which sulfatase activities are completely absent [50]. Using a recombinant adeno-associated virus of serotype 9 (rAAV9 vector) encoding the *SUMF1* gene, it was found that combined intracerebral ventricular and systemic administration was superior

to either single administration directly into brain, or systemic. The combined treatment led to widespread activation of sulfatases and virtually complete clearance of glycosaminoglycans in the central nervous system and visceral [51].

REFERENCES

1. Austin JH, Armstrong D, Shearer L. Metachromatic form of diffuse cerebral sclerosis. V The nature and significance of low sulfatase activity: a controlled study of brain liver and kidney in four patients with metachromatic leukodystrophy (MLD). *Arch Neurol* 1965; **13**: 593.
2. Austin J. Studies in metachromatic leukodystrophy. XII Multiple sulfatase deficiency. *Arch Neurol* 1973; **28**: 258.
3. Basner R, Von Figura K, Glossl J *et al.* Multiple deficiency of mucopolysaccharide sulfatases in mucosulfatidosis. *Pediatr Res* 1979; **13**: 1316.
4. Al Aqeel A, Ozand PT, Brismar J *et al.* Saudi variant of multiple sulfatase deficiency. *J Child Neurol* 1992; **7**(Suppl.): S12.
5. Bateman JB, Philippart M, Eisenberg SJ. Ocular features of multiple sulfatase deficiency and a new variant of metachromatic leukodystrophy. *J Pediatr Ophthalmol Strabismus* 1984; **21**: 133.
6. Schmidt B, Selmer T, Ingendoh A, von Figura K. A novel amino acid modification in sulfatases that is defective in multiple sulfatase deficiency. *Cell* 1995; **82**: 271.
7. von Figura K, Schmidt B, Selmer T, Dierks T. A novel protein modification generating an aldehyde group in sulfatases – its role in catalysis and disease. *Bioessays* 1998; **20**: 505.
8. Dierks T, Schlotawa L, Frese MA *et al.* Molecular basis of multiple sulfatase deficiency, mucopolipidosis II/III and Niemann-Pick C1 disease – lysosomal storage disorders caused by defects of non-lysosomal proteins. *Biochim Biophys Acta* 2009; **4**: 710.
9. Ballabio A, Cosma MP, Pepe S *et al.* The multiple sulfatase deficiency gene SUMF1 and its therapeutic potential for sulfatase deficiencies. *Am J Hum Genet* 2003; **73**: 200.
10. Fraldi A, Zito E, Annunziata F *et al.* Multistep, sequential control of the trafficking and function of the multiple sulfatase deficiency gene product, SUMF1 by PDI, ERGIC-53 and ERp44. *Hum Mol Genet* 2008; **17**: 2610.
11. Soong B-W, Casamassima AC, Fink JK *et al.* Multiple sulfatase deficiency. *Neurology* 1988; **38**: 1273.
12. Artigalás OA, da Silva LR, Burin M *et al.* Multiple sulfatase deficiency: clinical report and description of two novel mutations in a Brazilian patient. *Metab Brain Dis* 2009; **24**: 493.
13. Hogan K, Matalon R, Berlow S *et al.* Multiple sulfatase deficiency: clinical radiologic electrophysiologic and biochemical features. *Neurology* 1983; **33**(Suppl. 2): 245.
14. Lansky LL, Hug G. Enzymatic and structural studies in mucosulfatidosis. *Pediatr Res* 1979; **13**: 421.
15. Burk R, Valle D, Thomas G *et al.* Multiple sulfatase deficiency (MSD): clinical and biochemical studies in two patients. *Am J Hum Genet* 1981; **33**: 73A (Abstr.).
16. Nevsimalova S, Elleder M, Smid F, Zemankova M. Multiple sulfatase deficiency in homozygotic twins. *J Inherit Metab Dis* 1984; **7**: 38.
17. Bharrucha BA, Naik G, Sawliwala AS *et al.* Siblings with the Austin variant of metachromatic leukodystrophy multiple sulfatidosis. *Indian J Pediatr* 1984; **51**: 477.
18. Burk RD, Valle D, Thomas GH *et al.* Early manifestations of multiple sulfatase deficiency. *J Pediatr* 1984; **104**: 574.
19. Harbord M, Buncic JR, Chuang SA *et al.* Multiple sulfatase deficiency with early severe retinal degeneration. *J Child Neurol* 1991; **6**: 229.
20. Raynaud EJ, Escourolle R, Baumann N *et al.* Metachromatic leukodystrophy. Ultrastructural and enzymatic study of a case of variant O form. *Arch Neurol* 1975; **32**: 834.
21. Vamos E, Liebaers I, Bousard N *et al.* Multiple sulfatase deficiency with early onset. *J Inherit Metab Dis* 1981; **4**: 103.
22. Tanaka A, Hirabayashi M, Ishii M *et al.* Complementation studies with clinical and biochemical characterizations of a new variant of multiple sulfatase deficiency. *J Inherit Metab Dis* 1987; **10**: 103.
23. Zafeiriou DI, Vargiami E, Papadopoulou K *et al.* Serial magnetic resonance imaging and neurophysiological studies in multiple sulphatase deficiency. *Eur J Paediatr Neurol* 2008; **12**: 190.
24. Meikle PJ, Grasby DJ, Dean CJ *et al.* Newborn screening for lysosomal storage disorders. *Mol Genet Metab* 2006; **88**: 307.
25. Fuller M, Tucker JN, Lang DL *et al.* Screening patients referred to a metabolic clinic for lysosomal storage disorders. *J Med Genet* 2011; **48**: 422.
26. Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. *J Am Med Assoc* 1999; **281**: 249.
27. Horwitz AL. Genetic complementation studies of multiple sulfatase deficiency. *Proc Natl Acad Sci USA* 1979; **76**: 6496.
28. Fedde K, Horwitz AL. Complementation of multiple sulfatase deficiency in somatic cells hybrids. *Am J Hum Genet* 1984; **36**: 623.
29. Eto Y, Wiesmann UN, Carson JH, Herschkowitz NN. Multiple sulfatase deficiencies in cultured skin fibroblasts: occurrence in patients with a variant form of metachromatic leukodystrophy. *Arch Neurol* 1974; **30**: 153.
30. Eto Y, Rampini A, Wiesmann U, Herschkowitz NN. Enzymic studies of sulphatases in tissues of the normal human and in metachromatic leukodystrophy with multiple sulphatase deficiencies: arylsulfatases in A, B and C cerebroside sulphatase psychosine sulphatase and steroid sulphatases. *J Neurochem* 1974; **23**: 1161.
31. Chang PL, Rosa NE, Ballantyne ST, Davidson RG. Biochemical variability of arylsulfatases-A, -B and -C in cultured fibroblasts from patients with multiple sulphatase deficiency. *J Inherit Metab Dis* 1983; **6**: 167.
32. Steckel F, Hasilik A, Von Figura K. Synthesis and stability of arylsulfatase A and B in fibroblasts from multiple sulfatase deficiency. *Eur J Biochem* 1985; **151**: 141.
33. Eto Y, Gomibuchi I, Umezawa F, Tsuda T. Pathochemistry pathogenesis and enzyme replacement in multiple sulfatase deficiency. *Enzyme* 1987; **38**: 273.

34. Fluharty AL, Stevens RL, Davis LL *et al.* Presence of arylsulfatase A (ARSA) in multiple sulfatase deficiency disorder fibroblasts. *Am J Hum Genet* 1978; **30**: 249.
35. Eto Y, Tokoro T, Liebaers I *et al.* Biochemical characterization of neonatal multiple sulfatase deficiency (MSD) disorder cultured skin fibroblasts. *Biochem Biophys Res Commun* 1982; **106**: 429.
36. Patrick AD, Young E, Ellis C, Rodeck CH. Multiple sulphatase deficiency: prenatal diagnosis using chorionic villi. *Prenat Diagn* 1988; **8**: 303.
37. Steinmann B, Mieth D, Gitzelmann R. A newly recognized cause of low urinary estriol in pregnancy: multiple sulfatase deficiency of the fetus. *Gynecol Obstet Invest* 1981; **12**: 107.
38. Eto Y, Tokoro T, Ito F. Chemical compositions of acid mucopolysaccharides in urine and tissues of patients with multiple sulphatase deficiency. *J Inherit Metab Dis* 1981; **4**: 161.
39. Horwitz AL, Warshawsky L, King J, Burns G. Rapid degradation of steroid sulfatase in multiple sulfatase deficiency. *Biochem Biophys Res Commun* 1986; **6**: 26.
40. Steckel F, Hasilik A, von Figura K. Multiple sulfatase deficiency: degradation of arylsulfatase A and B after endocytosis in fibroblasts. *Eur J Biochem* 1985; **151**: 147.
41. Rommerskirch W, von Figura K. Multiple sulfatase deficiency: catalytically inactive sulfatases are expressed from retrovirally introduced sulfatase cDNAs. *Proc Natl Acad Sci USA* 1992; **89**: 2561.
42. Settembre C, Arteaga-Solis E, Ballabio A, Karsenty G. Self-eating in skeletal development: implications for lysosomal storage disorders. *Autophagy* 2009; **5**: 228.
43. Cosma MP, Pepe S, Annunziata I *et al.* The multiple sulfatase deficiency gene encodes an essential and limiting factor for the activity of sulfatases. *Cell* 2003; **113**: 445.
44. Dierks T, Schmidt B, Borissenko LV *et al.* Multiple sulfatase deficiency is caused by mutations in the gene encoding the human C-alpha-formylglycine generating enzyme. *Cell* 2003; **113**: 435.
45. Yiş U, Pepe S, Kurul SH *et al.* Multiple sulfatase deficiency in a Turkish family resulting from a novel mutation. *Brain Dev* 2008; **30**: 374.
46. Schlotawa L, Ennemann EC, Radhakrishnan K *et al.* SUMF1 mutations affecting stability and activity of formylglycine generating enzyme predict clinical outcome in multiple sulfatase deficiency. *Eur J Hum Genet* 2011; **19**: 253.
47. Buono M, Visigalli I, Bergamasco R *et al.* Sulfatase modifying factor 1-mediated fibroblast growth factor signaling primes hematopoietic multilineage development. *J Exp Med* 2010; **207**: 1647.
48. Plati T, Visigalli I, Capotondo A *et al.* Development and maturation of invariant NKT cells in the presence of lysosomal engulfment. *Eur J Immunol* 2009; **39**: 2748.
49. Ballabio A. Disease pathogenesis explained by basic science: lysosomal storage diseases as autophagocytic disorders. *Int J Clin Pharmacol Ther* 2009; **47**(Suppl. 1): S34.
50. Spanpanato C, De Leonibus E, Dama P *et al.* Efficacy of a combined intracerebral and systemic gene delivery approach for the treatment of a severe lysosomal storage disorder. *Mol Ther* 2011; **19**: 860.
51. Schlotawa L, Steinfeld R, von Figura K *et al.* Molecular analysis of SUMF1 mutations: stability and residual activity of mutant formylglycine-generating enzyme determine disease severity in multiple sulfatase deficiency. *Hum Mutat* 2008; **29**: 205.
52. Settembre C, Annunziata I, Spanpanato C *et al.* Systemic inflammation and neurodegeneration in a mouse model of multiple sulfatase deficiency. *Proc Natl Acad Sci USA* 2007; **104**: 4506.

MISCELLANEOUS

103. Congenital disorder of glycosylation, type Ia	781
104. Other forms of congenital disorders of glycosylation	787
105. α_1 -Antitrypsin deficiency	803
106. Canavan disease/aspartoacylase deficiency	811
107. Ethylmalonic encephalopathy	819
108. Disorders of creatine synthesis or transport	827

Congenital disorders of glycosylation, type Ia

Introduction	781	Treatment	785
Clinical abnormalities	781	References	785
Genetics and pathogenesis	784		

MAJOR PHENOTYPIC EXPRESSION

Infantile failure to thrive, inability to aliment orally, developmental delay, hypotonia, inverted nipples, esotropia, and an unusual lipodystrophy in which a general decrease in subcutaneous fat is associated with accumulated large fat pads in unusual sites, such as above the buttocks; pericardial effusions, hepatic dysfunction and pontocerebellar hypoplasia; in childhood, ataxia and disequilibrium, retinitis pigmentosa and stroke-like episodes; teenage neuropathy, muscular atrophy and secondary skeletal deformities; adult hypogonadism; deficient or absent carbohydrate moieties of secretory glycoproteins, especially serum transferrin; and deficient activity of phosphomannomutase.

INTRODUCTION

Twin girls with impaired psychomotor development and strabismus were reported in 1978 by Jaeken and colleagues [1] to have decreased amounts of thyroxin-binding globulin (TBG) in the serum and increased activity of serum arylsulfatase A. It was recognized that this unusual association was with two quite different glycoproteins, and heterogeneity was then found in transferrin of serum and cerebrospinal fluid [2]. It was hypothesized that the defect in common was in the carbohydrate moiety. Confirmatory evidence was the demonstration of deficiency of sialic acid, galactose, and N-acetylglucosamine in transferrin and in several other serum glycoproteins [3, 4].

More than 30 patients had been reported by 1993 [5] and more than twice that number had been identified in

Scandinavia alone [6]. At this time, hundreds of patients have been identified worldwide. The disorder was originally described in Belgium [1], but patients have been recognized in Spain [7], Taiwan [8], and the United States [9], including some of African ancestry [10]. Several related disorders of glycosylation have been discovered ([Chapter 105](#)). The fundamental biochemical abnormality in type Ia is in the activity of phosphomannomutase-2 (EC 5.4.2.28) ([Figure 103.1](#)) [11, 12].

CLINICAL ABNORMALITIES

Different phenotypic manifestations characterize this disorder at different ages. They have been differentiated [5] as:

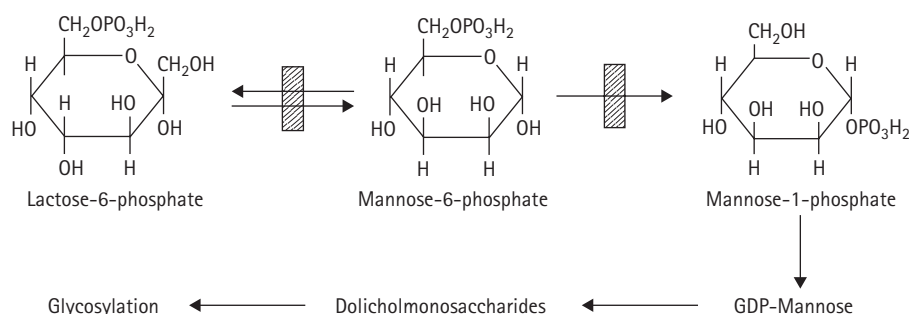


Figure 103.1 The phosphomannomutase reactions (pmm) and phosphomannose isomerase (PMI) and its relation to glycosylation.

- I. infantile multisystem stage;
- II. childhood ataxia-mental impairment stage;
- III. teenage leg atrophy stage;
- IV. adult hypogonadal stage.

Most patients have been born at term after an uneventful pregnancy, and birth weight has usually been normal. Some features may be recognized at birth: inversion of the nipples (Figures 103.2 and 103.3) is nearly universal (Table 103.1) and so is esotropia [5, 6]. Lipoatrophy may be manifest in a general reduction of subcutaneous tissue mass [6], or in lipoatrophic streaks or patches [5]. The most unusual feature is the occurrence of fat pads – collections of subcutaneous tissue in unusual places, most typically over or above the buttocks, but elsewhere too (Figures 103.4, 103.5, 103.6, and 103.7). Patches of thickened skin, especially on the legs, have been described as like tallow

Table 103.1 Inverted nipples: differential diagnosis

Congenital disorders of glycosylation
Bioppterin synthesis disorders
Citrullinemia (Chapter 31)
Isolated autosomal dominant (McKusick No. 163600)
Menkes disease (Chapter 74)
Methylmalonic acidemia (Chapter 3)
Molybdenum cofactor deficiency
Propionic acidemia (Chapter 2)
Pyruvate carboxylase deficiency (Chapter 48)
Very long chain acyl coenzyme A dehydrogenase deficiency (Chapter 41)
Weaver syndrome



Figure 103.2 HS: An eight-month-old infant with carbohydrate-deficient glycoprotein disease. Illustrated are the inverted nipples that constitute an early sign of the syndrome. There were lipoatrophic changes of the lower extremities. (This and the other pictures of patients with this disease kindly provided by Dr J Jaeken of the University of Louven, Belgium.)



Figure 103.3 HS: At eight months, showing close up of the inverted nipples.

or peau d'orange (orange peel) [5]. These features may be absent in the early months, in which the only manifestation may be failure to thrive. Alternatively, there may be early neonatal hypotonia, lethargy, edema, or cardiac failure [13].

Difficulties with feeding and failure to thrive regularly characterize the first three months. These infants display little interest in nursing, and nasogastric feedings have been required throughout the first year [6]. Frequent and projectile vomiting has been a problem. At one year, most patients have just doubled the weight of birth. Linear growth is also behind. Even older children may not chew, and they may gag and choke on textured or lumpy foods. However, notable exceptions have been observed, and macrosomia may be found [14].

Dysmorphic facial features described have included a high nasal bridge, prominent jaw, and large external ears (Figure 103.8) [5]. Some have had limitation of joint mobility in the legs. Head circumference at birth is normal, but microcephaly develops in about half of the patients [5].

Hypotonia or floppiness is regularly observed, and developmental delay is obvious early. Head lag may be seen as late as 12 months. In addition, some infants have had stroke-like episodes or episodes of acute deterioration in which developmental landmarks achieved have been lost. A few have had seizures. Ataxia is recognizable as early as seven months and imaging of the central nervous system reveals cerebellar and pontine atrophy, which may appear, especially after episodes of acute exacerbation. Hepatomegaly is a regular feature. Blood levels of transaminases are increased and levels of albumin and



Figure 103.4 HS: This figure illustrates the characteristic fat pads on the buttocks.



Figure 103.5 SF: A nine-month-old patient with the characteristic fat pads over the buttocks.



Figure 103.6 SF: This figure shows a close up of the fat pads.



Figure 103.7 HS: At eight months illustrating that fat deposits may occur elsewhere.

coagulation factors are decreased. There may be intestinal bleeding. Enlarged kidneys may be demonstrated by ultrasound or other forms of imaging.

Recurrent episodes of pericardial effusion have been seen commonly in infancy, and death from cardiac tamponade has been recorded [5]. There may be cardiomyopathy. Serious infections are also common and an infantile and early childhood mortality of 15–20 percent reflects predominantly infectious disease.

The childhood period from three to 12 years of age [5, 13, 15] is characterized by ataxia and mental impairment (Figure 103.9). Some have dyskinetic or choreoathetotic episodes. Only a few patients have learned to walk. Most sit unsupported after two years; they ultimately learn to stand on tiptoe because of contractures [16]. Most learn to use

a wheelchair. Disequilibrium and impaired coordination are prominent. Motor impairment is uniformly seen but the degree of impaired mental development is variable: IQs have ranged from 40 to 60 [5]. Patients understand spoken words but few develop linguistic skills; they speak in staccato fashion. Intellectual regression has not been seen except following stroke-like episodes. Deep tendon reflexes in the lower extremities disappear at this stage, and peripheral neuropathy becomes evident [16]. Retinal degeneration and retinitis pigmentosa is progressive in most [17]. The stroke-like episodes are more prominent in childhood; there may be stupor or coma, and convulsions, as well as hemiplegia, usually with recovery in hours to days. Permanent hemiplegia has been associated with cerebral infarction. Two patients were blind for months



Figure 103.8 LA: At two months. The face was somewhat dysmorphic, the forehead prominent, the nasal bridge depressed and tip anteverted, and there was some micrognathia. The ears were relatively large.

after an episode. One patient had an arterial thrombosis in a hand [18].

The teenage years are dominated by progressive muscle atrophy and weakness, especially of the legs. This appears to be primarily due to lower motor neuron dysfunction. Nerve conduction velocity is reduced. Cerebellar ataxia and poor coordination continue. Skeletal deformities, kyphosis, scoliosis, and keel thorax appear to be consequences of muscle atrophy. The unusual fat pads may disappear during this period. Seizures occur in about 50 percent of patients [13], but frequency may decrease in adolescence. Hepatopathy may stabilize or disappear [13].

Hypogonadism may be recognized in this period or in adulthood. This appears especially common in females. It may be hypergonadotrophic [5], but testicular atrophy has been seen in a male. There may be intermittent elevation of prolactin, growth hormone, insulin or follicle stimulating hormone (FSH). Premature ageing may also be seen in young adults [5]. Adults are short, compressed, and bent into flexor deformities. Neurologic deficits seem to stabilize in adulthood.

Clinical laboratory evaluation may reveal proteinuria. There is often an intermittent thrombocytosis, with counts up to 800,000 per mm^3 . There may be hypoprothrombinemia and diminished factors IX and XI. Elevated transaminases may become normal with age. The serum albumin is usually low, and some have a hypo- β -lipoproteinemia. The stroke-like episodes and thrombotic disease have been associated with decreased levels of antithrombin III and other major inhibitors of coagulation [18]. TBG is reduced in 75 percent of patients. Cerebrospinal fluid protein may be elevated. The electroencephalogram (EEG) is usually normal. Nerve conduction velocity is reduced and the deficit increases with age. Histologic examination of the liver reveals some



Figure 103.9 LA: A three-year-old female, was developmentally impaired and had motor disability of the lower limbs. Posturing of the left hand is evident. Retinitis pigmentosa leads to loss of vision.

degree of steatosis. There may be lamellar lysosomal inclusions on electron microscopy [19].

GENETICS AND PATHOGENESIS

The biochemical characteristic of this syndrome is the presence of secretory glycoproteins that are deficient in their carbohydrate content. Terminal trisaccharides are characteristically missing. As a result, a number of glycoproteins become abnormal, including transport proteins, enzymes, hormones such as prolactin and FSH, and coagulation factors. In the initial series of patients, serum activity of arylsulfatase A was recognized to be elevated [1]. Because of the abnormal TBG, it would seem that patients should be detected in programs of neonatal screening for hypothyroidism, but that has not been widely encountered.

Among the most used tests for the diagnosis of this condition is the isoelectric focusing of serum transferrin [20, 21]. Half of this glycoprotein is found to lack two or four of its terminal sialic acid moieties. The normal transferrin of serum is predominantly tetrasialotransferrin, and there are small amounts of mono-, di-, tri-, penta-, and hexa-sialotransferrins; in the disease state, loss of negatively charged sialic acid causes a cathodal shift. Abnormal transferrin is also present in liver and cerebrospinal fluid. Qualitative diagnosis is made by isoelectric focusing and

immunofixation of transferrin. Quantitative determination of carbohydrate-deficient transferrin indicated an approximately ten-fold elevation of cathodal transferrin forms [20]. Electrophoresis reveals low molecular weight isoforms of many serum glycoproteins, including α -1 antitrypsin [21]. The diagnostic accuracy may be improved using isoelectric focusing of α -1 antitrypsin and α -1 antichymotrypsin [22], and methodologies such as high performance liquid chromatography (HPLC) [23] and capillary zone electrophoresis [24] may be better suited to automation. Recently, the feasibility of tandem mass spectrometry has been demonstrated to elucidate the glycosylation of transferrin [25], an approach which allows for quantitative results and which offers the specificity to detect variant forms with more subtle differences in glycan processing [26].

The fundamental defect is in the synthesis and transfer of nascent dolichol-linked oligosaccharide precursors, and incorporation of labeled mannose into glycoproteins and the dolichol-linked oligosaccharide precursor is also shown to be deficient [27]. The abnormality in lipid-linked oligosaccharide biosynthesis could lead to failure to glycosylate sites on proteins and to abnormalities in glycoprotein processing or function. Ultrastructural studies of fibroblasts, Schwann cells, and hepatocytes have revealed membrane and lamellar, fibrillary and multi-vacuolated inclusions, suggesting a defect in macromolecular catabolism [15]. Furthermore, there is probably a cellular response to unfolded proteins that plays a part in the pathogenesis in this class of disease [28].

Neonatal screening for this disease has not been successfully implemented. While it was reported that the transferrin abnormality may be detectable in dried blood spotted on paper [13], it was not evident in a 19-week fetus [21]. It has been recommended that testing of transferrin glycosylation not be performed before 3 weeks of age to avoid false-negative results [29]. Of course, the disease is autosomal recessive, and although some heterozygotes may be recognizable chemically, heterozygote detection is not reliable.

The defect in phosphomannomutase [11, 12] (Figure 103.1) can be directly assayed in fibroblasts or leukocytes. In 16 patients, leukocyte activity ranged from 0.02 to 0.08 mU/mg protein as compared with the control range of 1.6 to 2.3. In fibroblasts, the range was 0.1 to 1.4 in patients and 2.2 to 6.4 in controls. The gene for phosphomannose-2 [30], designated PMM2, is localized on chromosome 16p13.3–p13.2, spanning 51.5 kb in eight exons and coding for 246 amino acids. At this point, more than 76 mutations have been described, including 66 missense mutations. The disease is pan-ethnic, but different populations have their own set of mutations [31]. The most common mutations are R141H and F119L, accounting for approximately 37 and 17 percent of alleles, respectively; the R141H mutation is found in more than 75 percent of patients of Caucasian origin [32], and the combination R141H/F119L accounts for about 38 percent of Caucasian patients. The R141H

mutation has never been found in a homozygous state, presumably because that condition is incompatible with life. The F119L mutation has a clear founder effect in the Scandinavian population, and the R141H mutation is associated with a specific haplotype which points to a single ancient mutational event. The observed frequency of the R141H allele (one in 72) in normal populations of Netherlands and Denmark, and the observed frequency of that allele in the compound heterozygous state with other mutations, suggests the frequency of the disease in that population would be expected to be around 1 in 20,000. The incidence in that population, however, has been estimated to be more on the order of 1 in 80,000 [33].

TREATMENT

No effective treatment has been reported. Nasogastric feeding and the use of high caloric diets are helpful in infancy, and painstaking approaches to feeding are required through childhood.

REFERENCES

1. Jaeken J, Van der Schueren-Lodeweyckx M, Casaer P *et al*. Familial psychomotor retardation with markedly fluctuating serum prolactin, FSH and GH levels, partial TBG deficiency, increased arylsulphatase A and increased CSF protein: a new syndrome? *Pediatr Res* 1980; **14**: 179.
2. Jaeken J, van Eijk HG, van der HC *et al*. Sialic acid-deficient serum and cerebrospinal fluid transferrin in a newly recognized genetic syndrome. *Clin Chim Acta* 1984; **144**: 245.
3. Jaeken J, Eggermont E, Stibler H. An apparent homozygous X-linked disorder with carbohydrate-deficient serum glycoproteins. *Lancet* 1987; **2**: 1398.
4. Stibler H, Jaeken J. Carbohydrate deficient serum transferrin in a new systemic hereditary syndrome. *Arch Dis Child* 1990; **65**: 107.
5. Jaeken J, Stibler H, Hagberg B. The carbohydrate-deficient glycoprotein syndrome. A new inherited multisystemic disease with severe nervous system involvement. *Acta Paediatr Scand Suppl* 1991; **375**: 1.
6. Petersen MB, Brostrom K, Stibler H, Skovby F. Early manifestations of the carbohydrate-deficient glycoprotein syndrome. *J Pediatr* 1993; **122**: 66.
7. Briones P, Vilaseca MA, Schollen E *et al*. Biochemical and molecular studies in 26 Spanish patients with congenital disorder of glycosylation type Ia. *J Inher Metab Dis* 2002; **25**: 635.
8. Chu KL, Chien YH, Tsai CE *et al*. Carbohydrate deficient glycoprotein syndrome type Ia. *J Formos Med Assoc* 2004; **103**: 721.
9. Enns GM, Steiner RD, Buist N *et al*. Clinical and molecular features of congenital disorder of glycosylation in patients with type 1 sialotransferrin pattern and diverse ethnic origins. *J Pediatr* 2002; **141**: 695.

10. Tayebi N, Andrews DQ, Park JK *et al*. A deletion-insertion mutation in the phosphomannomutase 2 gene in an African American patient with congenital disorders of glycosylation-Ia. *Am J Med Genet* 2002; **108**: 241.
11. Van Schaftingen E, Jaeken J. Phosphomannomutase deficiency is a cause of carbohydrate-deficient glycoprotein syndrome type I. *FEBS Lett* 1995; **377**: 318.
12. Jaeken J, Besley G, Buist N *et al*. Phosphomannomutase deficiency is the major cause of carbohydrate-deficient glycoprotein syndrome type I. *J Inherit Metab Dis* 1996; **19**(Suppl.): 1.
13. Hagberg BA, Blennow G, Kristiansson B, Stibler H. Carbohydrate-deficient glycoprotein syndromes: peculiar group of new disorders. *Pediatr Neurol* 1993; **9**: 255.
14. Neumann LM, von Moers A, Kunze J *et al*. Congenital disorder of glycosylation type 1a in a macrosomic 16-month-old boy with an atypical phenotype and homozygosity of the N216I mutation. *Eur J Pediatr* 2003; **162**: 710.
15. Hagberg B, Blennow G, Stibler H. The birth and infancy of a new disease: the carbohydrate-deficient glycoprotein syndrome. In: Fukuyama Y, Suzuki Y, Kamoshita S, Cesaer P (eds). *Fetal and Perinatal Neurology*. Basel: Karger, 1992: 314–19.
16. Blennow G, Jaeken J, Wiklund LM. Neurological findings in the carbohydrate-deficient glycoprotein syndrome. *Acta Paediatr Scand* 1991; **51**: 385.
17. Stromland K, Hagberg B, Kristiansson B. Ocular pathology in disialotransferrin developmental deficiency syndrome. *Ophthalmic Paediatr Genet* 1990; **11**: 309.
18. Iijima K, Murakami F, Nakamura K *et al*. Hemostatic studies in patients with carbohydrate-deficient glycoprotein syndrome. *Thromb Res* 1994; **76**: 193.
19. Conradi N, de Vos R, Jaeken J. Liver pathology in the carbohydrate deficient glycoprotein syndrome. *Acta Paediatr Scand* 1991; **375**: 50.
20. Stibler H, Jaeken J, Kristiansson B. Biochemical characteristics and diagnosis of the carbohydrate-deficient glycoprotein syndrome. *Acta Paediatr Scand* 1991; **375**: 21.
21. Harrison HH, Miller KL, Harbison MD, Slonim AE. Multiple serum protein abnormalities in carbohydrate-deficient glycoprotein syndrome: pathognomonic finding of two-dimensional electrophoresis? *Clin Chem* 1992; **38**: 1390.
22. Fang J, Peters V, Korner C, Hoffmann GF. Improvement of CDG diagnosis by combined examination of several glycoproteins. *J Inherit Metab Dis* 2004; **27**: 581.
23. Helander A, Bergstrom J, Freeze HH. Testing for congenital disorders of glycosylation by HPLC measurement of serum transferrin glycoforms. *Clin Chem* 2004; **50**: 954.
24. Jaeken J, Carchon H. Congenital disorders of glycosylation: a booming chapter of pediatrics. *Curr Opin Pediatr* 2004; **16**: 434.
25. Lacey JM, Bergen HR, Magera MJ *et al*. Rapid determination of transferrin isoforms by immunoaffinity liquid chromatography and electrospray mass spectrometry. *Clin Chem* 2001; **47**: 513.
26. Mills PB, Mills K, Mian N *et al*. Mass spectrometric analysis of glycans in elucidating the pathogenesis of CDG type IIx. *J Inherit Metab Dis* 2003; **26**: 119.
27. Powell LD, Paneerselvam K, Vij R *et al*. Carbohydrate-deficient glycoprotein syndrome: not an N-linked oligosaccharide processing defect, but an abnormality in lipid-linked oligosaccharide biosynthesis? *J Clin Invest* 1994; **94**: 1901.
28. Lecca MR, Wagner U, Patrignani A *et al*. Genome-wide analysis of the unfolded protein response in fibroblasts from congenital disorders of glycosylation type-I patients. *FASEB J* 2005; **19**: 240.
29. Marquardt T, Denecke J. Congenital disorders of glycosylation: review of their molecular bases, clinical presentations and specific therapies. *Eur J Pediatr* 2003; **162**: 359.
30. Matthijs G, Schollen E, Pardon E *et al*. Mutations in PMM2, a phosphomannomutase gene on chromosome 16p13, in carbohydrate-deficient glycoprotein type I syndrome (Jaeken syndrome). *Nat Genet* 1997; **16**: 88.
31. Matthijs G, Schollen E, Bjursell C *et al*. Mutations in PMM2 that cause congenital disorders of glycosylation, type Ia (CDG-Ia). *Hum Mutat* 2000; **16**: 386.
32. Schollen E, Kjaergaard S, Legius E *et al*. Lack of Hardy-Weinberg equilibrium for the most prevalent PMM2 mutation in CDG-Ia (congenital disorders of glycosylation type Ia). *Eur J Hum Genet* 2000; **8**: 367.
33. Kristiansson B, Stibler H, Hagberg B, Wahlstrom J. [CDGS-1 – a recently discovered hereditary metabolic disease. Multiple organ manifestations, incidence 1/80000, difficult to treat]. *Lakartidningen* 1998; **95**: 5742.

Other forms of congenital disorders of glycosylation

Introduction	787	Treatment	799
Clinical abnormalities	788	References	800
Genetics and pathogenesis	795		

MAJOR PHENOTYPIC EXPRESSION

Type I congenital disorders of glycosylation are defects in the initial assembly of dolichol-linked oligosaccharides, and accordingly result in glycoproteins which are deficient in entire oligosaccharide units; in transferrin, that translates to products which lack 2 or 4 sialic acid residues. Type II disorders are defects in the maturation and processing of glycoproteins in the Golgi system, resulting in transferrin forms which may have odd numbers of sialic acid units.

In general, Type I and Type II disorders present with varying degrees of psychomotor impairment, seizures, myopathy and hypotonia, hepatic dysfunction, edema, and/or coagulopathy. Notable specific features include:

Type Ib: Congenital hepatic fibrosis; protein-losing enteropathy, associated in some cases with coagulation abnormalities and/or hyperinsulinemic hypoglycaemia, transient elevations of aminotransferases, persistently low albumin; normal mental development.

Type If, Im, Iq: Ichthyosiform skin changes.

Type IIc, IIg: Prominent skeletal abnormalities.

Type IIc, IIh: Absence of sialyl Lewis X selectin ligand and leukocyte adhesion defect; Bombay blood type in Type IIc.

Since there are so many glycoprotein products involved in so many processes, the manifestations of these abnormalities ramify extensively and symptoms may show considerable variability and may also vary for a given patient as a function of age.

INTRODUCTION

In addition to the deficiency of phosphomannose mutase which characterizes CDG-Ia ([Chapter 104](#)), several other steps in the formation of glycoproteins have been identified [1]. For N-linked glycosylation, glycan assembly takes place first on the cytoplasmic side of the endoplasmic reticulum (ER), and then on the luminal aspect, resulting in the formation of the lipid-linked oligosaccharide dolichylpyrophosphate-GlcNAc₂Man₃Glc₃ ([Figure 104.1](#)). The oligosaccharide side-chain is then transferred en bloc to selected asparagines of nascent proteins. Then the glycan of the newly formed glycoprotein is processed in the ER, where the three glucoses are removed, and then in the Golgi, where the mannose-rich core is removed and replaced,

typically with two residues each of N-acetylglucosamine, galactose, and sialic acid ([Figure 104.2](#)).

The nomenclature has evolved in recent years. Since 1999 [2], the standard is to refer to these disorders as congenital disorders of glycosylation (CDG), replacing earlier terms such as carbohydrate-deficient glycoprotein syndromes (CDGS) and defects in the steps of N-glycosylation that take place in the cytoplasm or the endoplasmic reticulum as type I disorders. In these conditions, the manifestation is the absence of entire glycan side-chains. Accordingly, there is a distinctive pattern of glycosylation, which can be distinguished in glycoproteins such as transferrin, where the major expressed forms are missing two or four sialic acid residues. In contrast, the type II pattern also shows an increase of the trisialo- and monosialo-

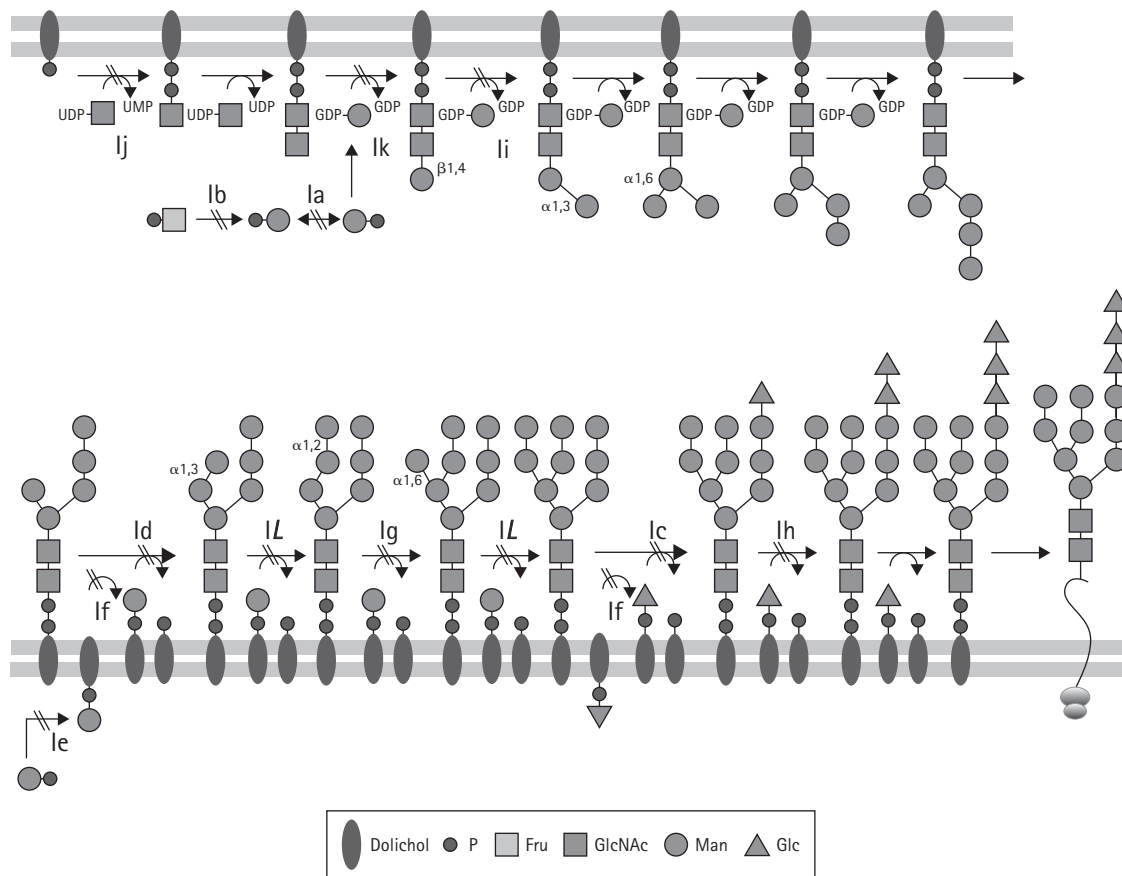


Figure 104.1 Type I disorders of N-linked glycosylation. Scheme shows the assembly of glycan chains beginning intraluminally (top line) and continuing extraluminally (lower line) until the transfer from the dolichol moiety to a nascent glycoprotein. Symbols for monosaccharides as shown: Man, mannose; Fru, fructose; Glc, glucose.

fractions, because of the incorporation of truncated or monoantennary sugar chains. Type II disorders arise from abnormalities in the processing of glycans in the Golgi apparatus. The standard in nomenclature [2] has been to sequentially assign letters to different specific enzyme disorders discovered in these pathways (giving, at the time of this writing, CDG-Ia through CDG-IL and CDG-IIa through CDG-IIe), and designating individual cases in which the specific defect has not been determined as CDG-Ix or CDG-IIx (Table 104.1).

There is a growing understanding of O-linked glycan metabolism, as well [3]. O-glycan biosynthesis begins in the Golgi apparatus, where GalNAc (or xylose in the case of glycosaminoglycans) is transferred to serine or threonine residues. O-glycans are less branched than most N-glycans, and they are generally found on mucins, but some proteins contain O-glycans either as short chains or as elongated bi-antennary structures [3]. Two categories of disease have been associated with disorders of O-linked glycan synthesis: xylosylprotein-4- β -galactosyltransferase deficiency, which is associated with the progeroid form of Ehler-Danlos syndrome (MIM 130070), and defects in exostosin (EXT1/EXT2 complex) which are associated with

multiple hereditary exostoses (MIM 133700, 133701). This chapter, however, deals only with the disorders of N-linked glycan metabolism other than CDG-Ia (Chapter 104), of which at least 16 forms have been discovered.

CLINICAL ABNORMALITIES

Type Ib. Phosphomannose isomerase deficiency (MIM 602579, 154550)

Since this form of CDG was elucidated in 1998 [4], at least 20 patients have been diagnosed [5]. In most, the presenting symptom was hepatic-intestinal disease [6] with liver fibrosis and protein-losing enteropathy, associated in some cases with coagulation abnormalities and/or hyperinsulinemic hypoglycemia [1]. The discovery of underlying defect in this group of patients stemmed from the recognition that the coagulopathy was associated with a profound deficiency of antithrombin III, which was known to be a common feature of CDG-Ia; isoelectric focusing of transferrin showed abnormalities similar to those in phosphomannose mutase (PMM2) deficiency, but

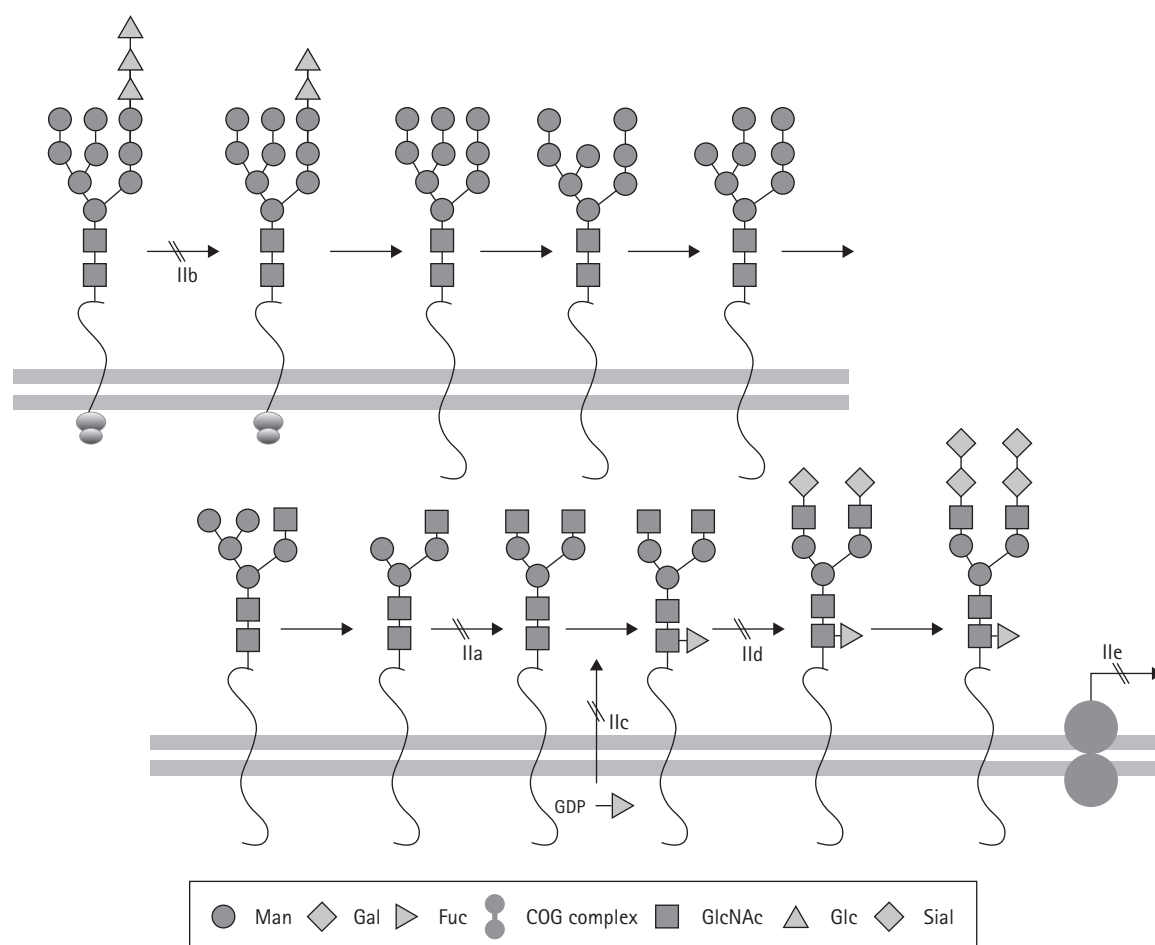


Figure 104.2 Type II disorders of N-linked glycosylation. Scheme shows the assembly of glycan chains beginning on the nascent glycoprotein in the endoplasmic reticulum and continuing on the protein within the Golgi apparatus. Symbols for monosaccharides as shown: Man, mannose; GlcNAc, N-acetylglucosamine; Gal, galactose; Glc, glucose; Fuc, fucose; Sial, sialic acid; COG complex, conserved oligomeric Golgi complex.

a defect in phosphomannose isomerase (Figure 104.3) was demonstrated [4].

In one consanguineous family with three affected siblings, the main feature was prolonged episodic vomiting, sometimes associated with diarrhea [4, 7]. Other notable findings included elevated aminotransferases during attacks and persistently low albumin. In one of those cases there had been an unexplained episode of multiorgan failure at two months. All three patients had normal mental and motor development. There are no dysmorphic features in this form of CDG (Figure 104.4). Similar histories and findings have been reported in other cases, but there have also been rather subtler, with episodic hepatic disease in infancy which disappeared after introduction of solid food [6], or a normal woman who had symptoms of hepatic fibrosis and recurrent venous thrombosis in childhood, and whose affected sibling died at the age of five years, but who was completely asymptomatic at age 33 when she was found

to have an abnormal transferrin glycosylation pattern and confirmed PMI mutation [8].

Type Ic. (MIM 603147, 604566)

Approximately 30 patients have been described with this form of CDG [1], originally described in 1998 in four members of a consanguineous Dutch family [9] (and previously designated type CDGS-V) [5]. The clinical presentation has been mainly neurologic, including moderate psychomotor impairment, axial hypotonia, strabismus, epilepsy in most, and ataxia in a few cases. The symptoms are generally milder than in CDG-Ia, and although specific physical findings and cerebellar hypoplasia are not regularly seen, there may be dysmorphic features and some abnormal distribution of fat (Figures 104.5, 104.6, and 104.7). Peripheral neuropathy has not been observed, nor has retinitis pigmentosa. Laboratory

Table 104.1 Defects in N-linked protein glycosylation

Type	Old name	Enzyme defect	Gene	OMIM	
Group I: Defects in the generation or transfer of the dolicholpyrophosphate-linked oligosaccharide					
Ia	CDGS Ia	Phosphomannomutase 2	<i>PMM2</i>	212065, 601785	16p13.3-p13.2
Ib	CDGS Ib	Phosphomannose Isomerase	<i>PMI</i>	154550, 602579	15q22-qter
Ic	CDGS V	Dolichyl-P-Glc:Man ₉ GlcNAc ₂ -PP-dolichyl α 1,3-Glucosyltransferase	<i>hALG6</i>	603147, 604566	1p22.3
Id	CDGS IV	Dolichyl-P-Man:Man ₉ GlcNAc ₂ -PP-dolichyl α 1,3-Mannosyltransferase	<i>hALG3</i>	601110	3q27
Ie	CDGS IV	Dolichol-P-Man synthase 1	<i>DPM1</i>	603503	20q13.13
If	–	Dolichol-P-Man Utilization Defect	<i>MPDU1</i>	604041	17p13.1-p12
Ig	–	Dolichyl-P-Man ₉ GlcNAc ₂ -PP-dolichyl α 1,6-Mannosyltransferase	<i>hALG12</i>	607143, 607144	22q13.33
Ih	–	Glc ₁ Man ₉ GlcNAc ₂ -PP-dolichyl α 3-glucosyltransferase	<i>hALG8</i>	608104, 608103	11q14
Ii	–	GDP-Mannose:Man ₁ GlcNAc ₂ -PP-dolichyl α 1,3-Mannosyltransferase	<i>hALG2</i>	607906, 607905	9q22
Ij	–	UDP-GlcNAc:dolichol-P GlcNAc-1-phosphate transferase	<i>DPAGT1</i>	608093, 191350	11q23.3
Ik	–	β 1,4-mannosyltransferase	<i>hALG1</i>	608540, 605907	16p13.3
IL	–	α 1,2-mannosyltransferase	<i>hALG9</i>	608776, 606941	11q23
Im	–	Dolichol kinase	<i>TMEM15</i>	610768, 610746	9q34.11
In	–	Flippase	<i>RFT1</i>	612015, 611908	3p21.1
Io	–	Dolichyl-phosphate mannosyltransferase	<i>DPM3</i>	612937, 605951	1q22
Ip	–	Endoplasmatic mannosyltransferase	<i>ALG11</i>	613661, 613666	13q14.3
Iq	–	Polyprenol reductase	<i>SRD5A3</i>	612379, 611715	4q12
Ix	–	Unknown genetic basis			
Group II: Defects in processing of N-glycans					
IIa	CDGS II	UDP-GlcNAc: α 6-D-mannoside β 1,2-GlcNAc transferase II (GnT II)	<i>MGAT2</i>	212066, 602616	14q21
IIb	–	α 1,2-Glucosidase I	<i>GCS1</i>	606056, 601336	2p13-p12
IIc	LAD II	GDP-fucose transporter I	<i>FUCT1</i>	266265, 605881	11
IId	–	UDP-Gal:N-acetylglucosamine β 1,4-galactosyltransferase I	<i>B4GALT1</i>	607091, 137060	9p13
IIe	–	Conserved oligomeric Golgi complex subunit 7	<i>COG7</i>	608779, 606978	16p
IIf	–	CMP-sialic acid transporter	<i>SLC35A1</i>	603585, 605634	6q15
IIg	–	Component of Oligomeric Golgi Complex 1	<i>COG1</i>	611209, 606973	17q25.1
IIh	–	Component of Oligomeric Golgi Complex 8	<i>COG8</i>	611182, 606975	16q22.1
IIj	–	Component of Oligomeric Golgi Complex 4	<i>COG4</i>	613489, 606976	16q22.1
IIx	–	Unknown genetic basis			

findings associated with CDG-Ia are also absent, including hypoalbuminemia and proteinuria. The glycosylation pattern of transferrin is the same as in the other type I conditions, but analysis of the lipid-linked oligosaccharides in fibroblasts reveals accumulation of Man₉GlcNAc₂ linked to dolichylpyrophosphate.

Type Id. (MIM 601110)

Two infants were described who were initially classified as type IV CDGS [10], with clinical features including postnatal

microcephaly, atrophy of the brain and corpus callosum, optic atrophy, iris coloboma, severe epilepsy with hypsarrhythmia, and failure of psychomotor development. There were no signs of liver dysfunction. Several blood glycoproteins demonstrated abnormal isoforms, including transferrin, alpha 1-antitrypsin, antithrombin and thyroxine-binding globulin, and the isoelectric focusing of serum transferrin showed a type 1 pattern but with a deficiency of 1 or 2 sialic acid residues, and without increase of asialotransferrin. Later analysis of lipid-linked oligosaccharide in fibroblasts from one of these patients showed an accumulation of the dolichylpyrophosphate-GlcNAc₂Man₉ precursor [11].

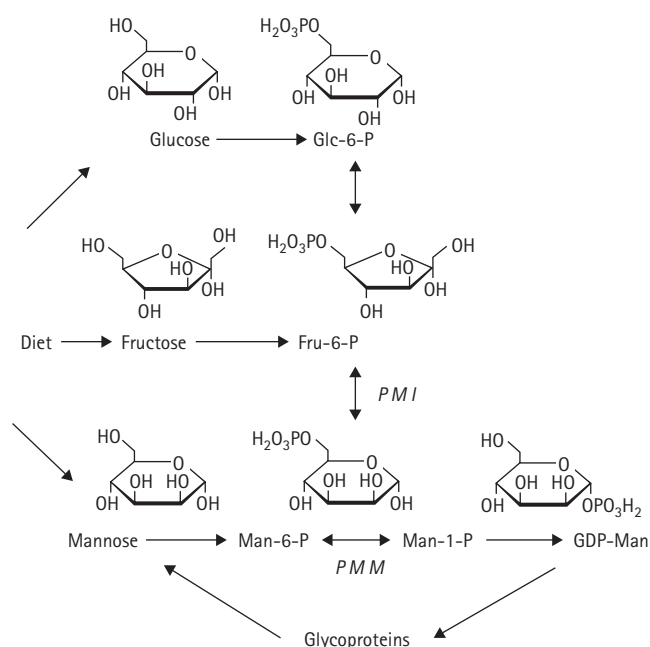


Figure 104.3 Chemical relations of mannose. The phosphomannose isomerase reaction which is defective in CDG-Ib.



Figure 104.4 BJ: A girl with CDG-Ib. As typical for these patients, she was cognitively normal and had no dysmorphic features. (Illustration kindly provided by Dr Hudson Freeze of the Sanford-Burnham Institute, San Diego.)

Type Ie. (MIM 608799, 603503)

Five cases have been reported. Four children from three families were described [12, 13] with very profound psychomotor impairment, severe epilepsy, hypotonia, failure to thrive, and mild dysmorphic features including



Figure 104.5 CMW: A mildly affected patient with CDG-Ic. (Illustration was kindly provided by Dr Hudson Freeze of the Sanford-Burnham Institute, San Diego.)

hypertelorism, high arched palate, small hands with dysplastic nails, and inverted nipples in only one case. Brain magnetic resonance imaging (MRI) showed delayed myelination in all, and frontal lobe atrophy in three, plus cerebellar atrophy in one. A fourth patient was found with milder symptoms [14]; she was a nine-year-old girl who had developed simple language and social skills, but exhibited drooling, ataxia, intention tremor and dyscoordination, and a history of a deep vein thrombosis. In that patient, the MRI showed delayed myelination and transitory hypodensities in the basal ganglia. Serum creatine phosphokinase was mildly to moderately elevated in all five cases, and transaminases were increased in the more severe cases. Isoelectric focusing of serum transferrin showed a type 1 pattern, but with only little or no increase of asialotransferrin. Only two patients have been identified in the United States (Figure 104.8).

Type If. (MIM 604041)

Two reports appearing simultaneously in 2001 presented four patients with this form of CDG. One patient displayed hypotonia and contractures at birth [15], developed nystagmus at three months with cortical blindness diagnosed by six months, seizures after five months, generalized cerebral atrophy, and had a scaling dry erythroderma which was initially hyperkeratotic, and intermittent bouts of vomiting without diarrhea. He developed limited, monosyllabic speech at two years, but subsequently lost the ability. At ten years, he could sit independently but could not stand or walk without support. Coagulation values were normal except for a reduced antithrombin III activity of 50 percent. Transferrin isoelectric focusing gave a typical type 1 pattern.



Figure 104.6 Patient MT (CDG-Ic) at nine months, demonstrating dysmorphic features and abnormal fat distribution. (Photographs kindly provided by Dr Hudson Freeze of the Sanford-Burnham Institute, San Diego.)

The three unrelated patients in the other report [16] had some similar features. One was a boy with intractable seizures from birth and no psychomotor development, who developed a widespread patchy desquamation, and recurrent apnea and ascites progressing to anasarca over the months before his death at age ten months. Another was a girl with a severe congenital ichthyotic skin disorder, psychomotor impairment, and attacks of hypertonia. At



Figure 104.7 Patient MT (CDG-Ic) at age three years. She had developed the ability to walk with support, but still had significant ataxia and dyscoordination.



Figure 104.8 PY (CDG-Ie), at age eight years. (Illustration was kindly provided by Dr Hudson Freeze of the Sanford-Burnham Institute, San Diego.)

age 16 years, she had severe growth failure, a developmental level of one year, and a persistent skin disorder. She also had a low cholesterol level, which was not apparently a feature in the other patients, and electron microscopy of a liver biopsy showed lamellar lysosomal inclusions [1]. The third child was a boy who had severe psychomotor impairment (developmental age 2.4 at ten years), but normal somatic growth, and no dermatologic disorder.

Type Ig. (MIM 607143, 607144)

Two cases were described in 2002. A girl born of consanguineous parents presented at birth with generalized

hypotonia and unspecified facial dysmorphism, and normal routine chemistry results except for hypocalcemia. She exhibited severe developmental delay, major hypotonia, failure to thrive, progressive microcephaly, and frequent upper respiratory infections with low IgG. At 18 months, a gastrostomy was placed, but neuroimaging of the brain was reportedly normal [17]. The other patient [18] was a male child delivered at 34 weeks in a pregnancy notable for HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets), with a neonatal course complicated by respiratory distress, hypoglycemia, feeding problems, and lethargy. At 16 months, computed tomographic (CT) scan showed evidence of slight frontal atrophy. Impaired development and hypotonia were present and at 2.5 years he was unable to sit. Dysmorphic features included inverted nipples, epicanthus, micropenis and cryptorchidism, club foot and wide 'sandal gaps.' Antithrombin-III was low, as were IgA, IgG, and IgM levels.

Type Ih. (MIM 608104, 608103)

A female patient was described in 2003 [19] with ascites and edema, severe hypoalbuminemia, diarrhea, and protein-losing enteropathy, no dysmorphic features, and normal development through 18 months. A trial of oral mannose was unsuccessful, but diarrhea resolved after 18 months of a low fat diet supplemented with essential fatty acids. Three other patients from two families were reported [20] with severe presentations and early death. A brother and sister had intrauterine growth impaired and reduced fetal movements. The boy was lethargic postnatally, with diarrhea, vomiting, and ascites, and death at three months. The girl developed edema and electrolyte abnormalities within hours of birth and expired at 3 days. The third patient had dysmorphic features including an asymmetric skull, large fontanelle, hypertelorism, low-set ears, long philtrum, short neck, cryptorchidism, camptodactyly, club feet, bilateral thoracic and pulmonary hypoplasia, perimembranous and trabecular ventricular septal defects, patent ductus arteriosus, multiple cystic intra- and extra-hepatic bile ducts, cholestasis, and diffuse renal microcysts. He had no diarrhea or vomiting, but developed progressive ascites and died at three months.

Type Ii. (MIM 607906, 607905)

A single patient was described in 2003 [21], a German girl who was found at two months of age to have bilateral colobomas of the iris and a unilateral cataract. Despite replacement of the affected lens, her vision remained poor and nystagmus developed. Seizures and hypersarrhythmia developed after four months, and MRI at five months showed delayed myelination, with no progression of myelination by eight months. Laboratory tests were normal except for prolonged activated partial thromboplastin time and markedly reduced level of factor XI.

Type Ij. (MIM 608093, 191350)

An infant was presented in 2003 [22] who developed infantile spasms within 72 hours of immunization at four months and went on to display microcephaly, continued intractable seizures, and significant developmental delay, with rudimentary language at six years. She also had a few minor dysmorphic features, including a high-arched palate, fifth finger clinodactyly, single palmar creases, and dimples on the upper thighs.

Type Ik. (MIM 608540, 605907)

Three patients were described in 2004. One [23] had intrauterine evidence of nonimmune hydrops and hepatosplenomegaly. He was born at 35 weeks with multiple dysmorphic features, including a large fontanelle, hypertelorism, micrognathia and hypogonadism, and displayed contractures, cardiomyopathy, areflexia, and multifocal epileptic activity. Two others [24] also presented in infancy with severe seizures, microcephaly and cerebral atrophy, and significant coagulation abnormalities. One of these patients developed seizures at five months, had frequent unexplained febrile episodes, developed progressive stupor, and expired at ten months. The other had seizures starting at 2 hours of life, developed a nephrotic syndrome at one month, was noted to have a severe paucity of circulating B cells and no detectable IgG, developed progressive stupor and died of respiratory failure at 11 weeks.

Type IL. (MIM 608776, 606941)

One patient was reported in 2004 [25] with microcephaly, central hypotonia, seizures, developmental delay, hepatomegaly, and bronchial asthma.

Type Im. (MIM 610768, 610746)

Two consanguineous families were described in 2007 [26]. Two affected infants were described in each family. Notable abnormalities included ichthyosis and cardiomyopathy.

Type In. (MIM 612015, 611908)

This defect was confirmed [27] in a patient with symptoms including marked developmental delay, hypotonia, seizures, hepatomegaly, and coagulopathy.

Type Io. (MIM 612937, 605951)

A 27-year-old woman with proximal muscle weakness and low-normal IQ was described in 2009 [28]. She had

a history of muscle weakness and cardiomyopathy, with muscle biopsy findings of fiber size variation, internal nuclei, necrotic fibers, rimmed vacuoles, and interstitial fibrosis; there was also a stroke-like episode.

Type Ip (MIM 613661, 613666)

Two consanguineous siblings were reported [29] with hypotonia, seizures, psychomotor impairment, and death by age 2 years. Dysmorphic features including microcephaly, low hairline, and inverted nipples were noted in one.

Type Iq (MIM 612379, 611715)

A number of consanguineous families have been shown [30] to have a type I CDG underlying a constellation of eye malformations (including coloboma or optic disc hypoplasia), variable visual loss, brain abnormalities (including cerebellar atrophy or vermis malformations), hypotonia, psychomotor impairment, and facial dysmorphism. Ichthyosiform erythroderma or congenital heart defects were present in some patients, as well as microcytic anemia, elevated liver enzymes, decreased antithrombin III and coagulopathy.

Type IIa. (MIM 212066, 602616)

Jaeken and Matthijs [1] summarized the presentation of the first three patients with this form of CDG, characterized by severe impaired mental development, dysmorphic features, epilepsy and striking stereotypic behavior. CDG-IIa was recognized (and initially called CDGS-II) as early as 1991 in an Iranian girl [31] and soon after in a Belgian boy [32], because of lowered serum values of a number of glycoproteins, similar to those with CDG-Ia. However, unlike several defects in the CDG-I group, no lysosomal inclusions were seen on liver biopsy, the appearance of the cerebellum was normal on MRI, and the pattern of transferrin isoelectric focusing was distinctive [33].

Type IIb. (MIM 606056, 601336)

A neonate was identified in 2000 [34], with generalized hypotonia and dysmorphic features including a prominent occiput, short palpebral fissures, long eyelashes, broad nose, retrognathia, high arched palate, and generalized edema, and a course notable for hepatomegaly, hypoventilation, seizures, and death at 74 days.

Type IIc. (MIM 266265, 605881)

Two unrelated Arab children from consanguineous families were reported in 1992 [35], with severely impaired

mental development, microcephaly, cortical atrophy, seizures, hypotonia, dwarfism, and recurrent infections with neutrophilia. The absence of the sialyl Lewis X (sLeX) ligand for the selectins and the absence of the H antigen in Bombay phenotype, both being manifestations of failure of fucosylation, a defect in fucose metabolism was hypothesized. The condition was designated Rambam-Hasharon syndrome, but because of the effects on selectin binding, as had been described the leukocyte adhesion defect (LAD) due to mutation of the integrin β -subunit, this condition was also termed LAD type II. Subsequently, another case in a patient of Turkish origin was ascertained [36], and two other Arab children were ascertained by the original group [37], with similar clinical features in all cases.

There is no abnormality of isoelectric pattern of transferrin, as sialylation is normal, and delayed separation of the umbilical cord, as seen in LAD type I, is not a regular feature. There were some interesting differences among the patients. The Arab patients had relatively mild infections, with the main manifestation being periodontitis, while the Turkish patient had more severe infections. Also, there was a clinical response to fucose supplementation in the Turkish child, with induction of core fucosylation of serum glycoproteins, disappearance of infections and fevers, and improvement of psychomotor capabilities. There was no apparent response to fucose supplementation in the Arab patients [38].

Type IIId. (MIM 607091, 137060)

A single patient was described in 2002 [39], a 16-month-old boy born of nonconsanguineous parents, presenting with hypotonia, myopathy with elevated creatine kinase, developmental delay, and coagulopathy. He also had progressive macrocephaly associated with a Dandy-Walker malformation, which might have been coincidental. The transferrin isoelectric pattern was abnormal, and there was also evidence of reduced sialic acid content of α 1-antitrypsin and α 1-antichymotrypsin.

Type IIe. (MIM 608779, 606978)

Two consanguineous siblings were described [40] with dysmorphic features including loose, wrinkled skin, low-set dysplastic ears, micrognathia, and short neck; in the male sibling, there was also failure of development of the humeral and tibial epiphyses, and the female sibling had short limbs. There were neurologic abnormalities including generalized hypotonia and seizures. Hepatosplenomegaly and progressive jaundice were observed, and the babies died at 5 and 10 weeks with recurrent infections and cardiac insufficiency.

Type IIIf. (MIM 603585, 605634)

A boy was described in 2001 [41] who presented with cutaneous hemorrhages and multiple episodes of bleeding,

including a massive spontaneous bleed in the posterior ocular chamber and severe pulmonary hemorrhage. He also had multiple recurrent bacterial infections, and despite bone marrow transplantation at age 34 months, the patient died of complications at age 37 months.

Type IIg (MIM 611209, 606973)

This disorder was first described [42] in a female infant with failure to thrive, rhizomelic short stature, hypotonia, psychomotor impairment, progressive microcephaly, and mild hepatosplenomegaly. Two other male patients were reported [43] with more severe features, and features of cerebrocostomandibular syndrome. Skeletal anomalies included fused ribs and posterior rib gaps, misaligned and malformed vertebrae, and talipes equinovarus. There was an enlarged cisterna magna, hypoplastic cerebellar vermis, and mild psychomotor impairment.

Type IIh (MIM 611182, 606975)

A female child was reported whose early development was normal before developing acute encephalopathy and loss of milestones at 6 months of age [44], with subsequent cerebral atrophy, cerebellar atrophy, ataxia, and seizures. There were episodic spontaneous hematomas and coagulopathy. A male patient was also described [45] with no acute episodes, but severe primary developmental deficits, hypotonia, muscular atrophy, hyporeflexia, and seizures; pathological features included chronic axonal neuropathy, and neurogenic changes on muscle biopsy.

Type IIi (MIM 613612, 606821)

A 14-year-old consanguineous Iraqi girl was described [46] with moderate mental impairment, inarticulate speech, ataxia, hypotonia, and pronounced atrophy of the cerebellum and brainstem. She had abnormal N-glycosylation of transferrin and alpha-1-acid glycoprotein, as well as decreased O-glycosylation of apolipoprotein C-III.

Type IIj (MIM 613489, 606976)

A boy was reported [47] who presented at 4 months with fever, irritability, and complex seizures after a vaccination. Mild dysmorphic features and nonspecific neurologic signs were reported, as well as increased serum transaminases and decreased platelets and coagulation factors. With time, microcephaly and frontotemporal cerebral atrophy became more evident, and he had moderate psychomotor impairment, somewhat less severe than in other described COG defects.

GENETICS AND PATHOGENESIS

The biochemical characteristic of this syndrome is the presence of secretory glycoproteins which are deficient in their carbohydrate content. As a result, a wide range of glycoproteins become abnormal, including transport proteins, enzymes, hormones such as prolactin and FSH, and coagulation factors such as antithrombin-III and factor XI. Among the most widely used tests for the diagnosis of this condition is the isoelectric focusing of serum transferrin [48]. Secondary abnormalities in which hypoglycosylation of transferrin is found include alcoholism and galactosemia. More refined electrophoretic techniques may be used to reveal lower molecular weight glycoforms of several serum glycoproteins in CDG, including α -1 antitrypsin and α -1 antichymotrypsin [49, 50]. Abnormal transferrin glycoforms may also be detected by high performance liquid chromatography (HPLC) [51] or capillary zone electrophoresis [52], techniques which are more suited to automation. Tandem mass spectrometry has been shown to be useful to study the glycosylation of transferrin in clinical samples [53], and that approach allows for quantitative results and can permit detection of subtle variants in glycan processing [54].

All known forms of CDG are autosomal recessive, and while biochemical changes may be discernable in heterozygotes in some cases, carrier detection is not reliable unless by direct testing for mutations. Neonatal screening for these diseases has not been shown to be feasible, and it has been recommended that testing of transferrin glycosylation not be performed before 3 weeks of age to avoid false-negative results [5].

Type Ib

The human mannose 6-phosphate isomerase (MPI) gene (designated PMI) encodes 423 amino acids in eight exons, spanning only 5 kb [7], and is located on chromosome 15. To date, 16 different mutations have been found in more than ten families [55, 56]. Thirteen of the mutations are missense mutations, mostly affecting conserved amino acids. There is one known case of a one-base insertion, and two nucleotide substitutions at splice sites.

There is no clear explanation for the clinical differences between CDG-Ib (PMI deficiency) and CDG-Ia (PMM2 deficiency), which, though the metabolic steps are consecutive, are strikingly different and particularly intriguing with respect to neurological manifestations. One possible explanation is that brain hexokinase, which can convert mannose to mannose 6-phosphate, has a rather high affinity for mannose, whereas glucokinase, the major hexokinase present in hepatocytes, has a very low affinity for mannose and is thus less efficient in bypassing PMI.

Type Ic

The primary defect is in the dolichylpyrophosphate-GlcNAc₂Man₅ α -1,3-glucosyltransferase which adds the first three glucose residues in the lipid-linked oligosaccharide (Figure 104.1), first identified in yeast as the ALG6 gene product. Nonglycosylated oligosaccharides are inefficiently transferred to proteins, and accordingly there is accumulation of Man₅GlcNAc₂ linked to dolichylpyrophosphate in fibroblasts [9]. The human ALG6 gene, *hALG6* has 14 exons and spans 55 kb on chromosome 1p22.3 [57]. The 1521 bp open reading frame encodes 507 amino acids. There is a frequent A333V mutation, which was homozygous in four related patients in the first cases [58] and an additional four unrelated patients in a later study [57], which also described a patient who was a compound heterozygote for A333V and an intron 3 5'-splice site transition IVS3C5G > A. A founder effect of the A333V mutation was demonstrated by haplotype analysis [57]. Four other missense mutations have been described [57, 59, 60], and it appears that one of these, F304S, may be a frequent polymorphism [1]. Overall, at the time of writing, five missense mutations, one splice site substitution, and two small deletions have been described [56].

Type Id

The defect was determined to involve the endoplasmic reticulum-associated mannosyltransferase that attaches a mannosyl residue from dolicholphosphomannose to Man₅GlcNAc₂-pyrophosphate-dolichol [11]. The human gene, identified as the Not 56-like protein gene (NOT56L), or the ortholog of the yeast ALG3 gene, codes for 438 amino acids and contains nine exons over 5 kb, and is located on chromosome 3q27. Two missense mutations have been found [11, 59], and a silent mutation in exon 1 has also been shown to give rise to an mRNA deletion due to a cryptic splice site [61].

Type Ie

Analysis of the lipid-linked oligosaccharides in the ER of fibroblasts revealed an accumulation of dolichylpyrophosphate-Man₅GlcNAc₂, consistent with a defect in the dolichol-phosphate-mannose synthase (Figure 104.1). The dolichol-phosphate-mannose synthase complex is composed of at least three proteins: a catalytic subunit (DPM1), a membrane-associated anchor (DPM2p), and a stabilizing subunit (DPM3) [62]. DPM is important in the biosynthesis of several glycoconjugates, as it is the donor not only of the last four mannoses in N-linked glycans (Figure 104.1), but also all three mannoses in glycosylphosphatidylinositols, and apparently also for some processes of O- and C-linked protein glycosylation. At this point, all of the recognized human defects in dolichol-phosphate-mannose

synthase have been found to be in DPM1, a gene of nine exons spanning 23.6 kbp, which encodes 260 amino acids [13]. Only four patients have been analyzed at the molecular level. One patient was homozygous for the missense mutation R92G [12], two others were compound heterozygous for R92G with either a 13 bp deletion (331–343del13) in exon 4 [12] or a single base deletion (628 delC) in exon 8 [13], and the fourth patient was homozygous for the missense mutation S248P [14].

Type If

Examination of the patients' fibroblast lipid-linked oligosaccharides showed accumulation of truncated forms, including Man₅GlcNAc₂-, Man₉GlcNAc₂-, but also of fully assembled Glc₃Man₅GlcNAc₂-linked forms. This indicated a defect in dolichol-phosphate-mannose utilization, and also in dolichol-phosphate-glucose utilization, as had been seen in the CHO-derived Lec35 mutant cells, and mutations were found in the Lec35/MPDU1 (mannose-phosphate-dolichol utilization defect 1) locus, also termed SL15 (suppressor of Lec15). As a result of this defect, there is also an expected deficiency in formation of glycosylphosphoinositols, and there is indirect evidence for defective GPI anchor formation [1]. The MPDU1 locus is found on chromosome 17p13.1–p12, spanning 4.34 kb with seven exons coding for 247 amino acids. One patient was homozygous for a L119P, another homozygous for a G73E missense mutation, and a third patient [16] was a compound heterozygote for a missense mutation, MIT, affecting the initiating methionine, and a one base frameshift deletion, 511delC. The fourth patient [15] was homozygous for an L74S missense mutation.

Type Ig

The presence of a truncated GlcNAc₂Man₇ N-linked oligosaccharide [18] suggested that there was a defect in the transfer of the last two mannose residues to the nascent glycan, and as there was no detectable untrimmed GlcNAc₂Man₇Glc₃ species in the presence of the inhibitor castanospermine, the defect was deduced to be in the transfer of the GlcNAc₂Man₇ substrate to the oligosaccharyltransferase complex (Figure 104.1). The human ortholog to the yeast ALG12 gene was identified *in silico*, and the cDNA sequence documented two amino acid substitutions leading to missense mutations T67M and R146Q in one patient [18], and a homozygous F142V mutation in the other [17]. The human ALG12 gene is localized on chromosome 22q13.33, with ten exons spanning 15 kb and coding for 488 amino acids.

Type Ih

The pattern of accumulated dolichol-linked oligosaccharides indicated a defect in the glucosyltransferase that

adds the second glucose residue [19], the dolichyl-P-glucose: Glc₁Man₉GlcNAc₂-PP-dolichyl α 3-glucosyltransferase (ortholog of yeast ALG8). The gene is located on chromosome 11q14 and consists of 13 exons coding for 526 amino acids, with 12 transmembrane domains, and an endoplasmic reticulum retention signal. The index case was found to be a compound heterozygote for two frame shift mutations in exon 4: a one base deletion, del413C, and a one base insertion, ins396A. The severely affected patients reported by Schollen *et al.* in 2004 [20] were compound heterozygotes for a splice site mutation and a missense mutation: in one family the combination was IVS1-2,A.G and T47P, and in the other family the combination was IVS6+4,A>G and G275D.

Type li

In addition to the normal dolichol-linked oligosaccharide, Glc₅Man₉GlcNAc₂, which is the normal form, although it was present at a slightly reduced level [21], cells from the index patient accumulated Man₁GlcNAc₂- and Man₂GlcNAc₂-PP-dolichol. As predicted, GDP-mannose:Man₁GlcNAc₂-PP-dolichol α 1,3-mannosyltransferase (the enzyme catalyzing the addition of the second mannose, and the ortholog of yeast ALG2) was defective (Figure 104.1). The gene is located on chromosome 9q22 and codes for a polypeptide of 416 amino acids. The index patient [21] was a compound heterozygote for a one base deletion, del1040G and a 393G>T substitution.

Type lj

Metabolic labeling of cultured fibroblasts from the index patient [22] revealed accumulation of the normal GlcNAc₂Man₉Glc₂ product bound to dolichol and to asparagine, but in reduced amounts. A specific assay of microsomal fractions for UDP-GlcNAc:dolichol phosphate N-acetyl-glucosamine-1-phosphate transferase demonstrated a deficiency of that enzyme (designated DPAGT1), which catalyzes the first step in N-linked oligosaccharide synthesis (Figure 104.1) and is inhibited by the antibiotic tunicamycin. The gene, located on chromosome 11q23.3, consists of nine exons spanning 5.13 kb, and codes for 408 amino acids. In the index case [22], a transition in exon 5 leading to the missense mutation Y170C was found on the paternal allele; though no mutation was found on the maternal allele, it produced only 12 percent of the normal amount of mature mRNA, the remainder showing a complex exon-skipping pattern.

Type lk

To analyze the early intermediates of N-linked glycan synthesis, HPLC was performed with fluorescence

detection of 2-amnobenamide-coupled oligosaccharides [51], and dolichylpyrophosphate-GlcNAc₂ was found, suggesting a deficiency of the β 1,4-mannosyltransferase homologous with the yeast ALG1, which performs the first mannosylation step (Figure 104.1). The gene, located on chromosome 16p13.3, codes for 464 amino acids. A homozygous missense mutation, S258L, was found in two unrelated patients [23, 24], and the same mutation was found as one allele in another patient who was a compound heterozygote for E342P [24].

Type ll

Lipid-linked oligosaccharides showed accumulation of GlcNAc₂Man₆ and GlcNAc₂Man₈ forms, and N-linked oligosaccharides included an abnormal GlcNAc₂Man₆ form [25], indicating a defect in the α 1,2-mannosyltransferase which adds the seventh and ninth mannose residues on the growing dolichol-linked oligosaccharide (Figure 104.1), previously identified in yeast as ALG9. The human ortholog of ALG9 had been described as DIBD1 (disrupted in bipolar disorder 1), a candidate gene associated with bipolar affective disorder, but shown by linkage analysis to have no evidence of a role in susceptibility to bipolar disorder [64]. The mannosyltransferase gene is located on chromosome 11q23, with 15 exons coding 611 amino acids with a multiple membrane spanning motif. An E523K missense mutation was found in the index patient [25].

Type lm

Homozygous mutations in the gene for dolichol kinase were found [26], consistent with the observation that transferrin glycosylation was markedly decreased, but structures which were formed were structurally normal.

Type ln

The accumulation of lipid-linked DolPP-GlcNAc(2)Man(5) and marked protein underglycosylation was consistent with yeast depleted for the Rft1 protein, the so-called “flippase” enzyme which catalyzes the translocation of Man(5)GlcNAc(2)-PP-Dol to the luminal side of the ER. Mutations in the RFT1 gene were demonstrated in a patient with CDG In [27].

Type lo

A homozygous missense mutation was identified in the DPM3 gene coding for a subunit of dolichyl-phosphate mannosyltransferase [28]. DPM activity is necessary for N-glycosylation, C-mannosylation, O-mannosylation, and GPI-anchor formation; the authors proposed that defective

O-mannosylation of alpha-dystroglycan accounted for the phenotype of muscular dystrophy, bridging the congenital disorders of glycosylation to the dystroglycanopathies.

Type Ip

Isoelectric focusing of serum transferrin from patient fibroblasts showed an increased amount of di- and asialo-transferrin with a decrease of tetrasialo-transferrin, consistent with CDG type I. Further studies showed an accumulation of shortened dolichol-linked oligosaccharides, indicating a defect at the step adding the fourth and fifth mannose residues. A homozygous missense mutation was identified [29] in the gene for ALG11, a mannosyltransferase that uses GDP-mannose to add the fourth and fifth mannose residues to growing dolichol-linked oligosaccharide side chains.

Type Iq

Biochemical analysis indicated [30] that the synthesis of the lipid carrier dolichol-phosphate (Dol-P) is reduced, and homozygous mutations were demonstrated in the SRD5A3 gene, a polyprenol reductase with similarities to steroid reductases.

Type IIa

Isoelectric focusing of serum transferrin showed a type 2 pattern but with nearly absent tetrasialotransferrin, and fine structure analysis of the glycans on serum transferrin revealed that some of the normal, disialo-biantennary N-glycans are replaced by truncated, monosialo-monoantennary N-glycans [27], whose structure indicated that the defect was in UDP-N-acetylglucosamine:alpha-6-D-mannoside-beta-1,2-N-acetylglucosaminyltransferase II (GlcNAc-transferase II, GnT II) in the Golgi (Figure 104.2). GnT II activity was reduced by over 98 percent in fibroblasts and mononuclear blood cells from patients [1]. The human GnT II gene (designated MGAT2) is located on chromosome 14q21, and the coding region contains only one exon, which encodes a protein of 447 amino acids. Mutation analysis of the GnT II coding sequence (MGAT2 gene) in the first two patients described revealed that they were each homozygous for missense mutations in the C-terminal catalytic domain, S290F and H262R [65]. A third patient [66] was a compound heterozygote for a missense (N318D) and a nonsense (C339X) mutation. A knockout mouse model for GnT II deficiency has been developed [67]. Homozygous knockout mice survive to term, but are born stunted with various congenital abnormalities and die early in the neonatal phase; this severity may explain the rarity with which this disorder is encountered in human cases [9].

Type IIb

Isoelectric focusing of transferrin was normal, but serum hexosaminidase showed a slight cathodal shift. A tetrasaccharide, identified as Glc(α1-2)Glc(α1-3)Glc(α1-3)Man, accumulated in the patient's urine. Electron microscopy showed lamellar intralysosomal inclusions in liver parenchymal cells and macrophages and empty, membrane-bound vacuoles in neurons of the frontal and occipital lobes of the brain. A defect in glucosidase-I, the first step of N-glycan processing (Figure 104.2) was demonstrated in liver and cultured fibroblasts [29]. The *GCS1* gene is located on chromosome 2p12-p13 and encodes 834 amino acids. The only known patient [29] was found to be a compound heterozygote for two missense mutations, R486T and F652L.

Type IIc

The clinical features arise from hypofucosylation of N- and O-linked glycoproteins. There is no abnormality in the isoelectric pattern of serum transferrin because the normal glycan in transferrin does not contain fucose, and there is no deficiency of sialylation. Cells from a CDG-IIc patient were shown to have decreased transport of GDP-fucose into the Golgi [68], and complementation cloning identified the defect in a GDP-fucose transporter [69, 70], which is designated FUTCT1. The gene has been assigned to chromosome 11 by radiation hybrid mapping. All of the Arab cases were homozygous for a T308R missense mutation in the predicted ninth membrane-spanning domain [71], consistent with a founder effect, and the Turkish patient was homozygous for R147C mutation in the fourth transmembrane domain [70].

Type IId

The isoelectric pattern of transferrin is abnormal [39], with strongly elevated tri-, di-, mono- and asialotransferrin bands (increasing in that order) and a markedly decreased tetrasialotransferrin, and there is also abnormal glycosylation of two other glycoproteins, α1-antitrypsin and α1-antichymotrypsin. The defect was determined [72] to be in the Golgi enzyme UDP-Gal:N-acetylglucosamine β-1,4-galactosyltransferase I (Figure 104.2). The gene for this galactosyltransferase, designated B4GALT1, is localized on chromosome 9p13, contains six exons spanning more than 50 kb, and encodes a predicted 400 amino acid protein with an N-terminal membrane-anchoring domain [73]. A knockout mouse model [74] exhibits impaired growth and semilethality, with excessive epithelial cell proliferation of the skin and small intestine, and abnormal differentiation in intestinal villi. Interestingly, the knock-out mice are reported to be deficient in the production of lactose, and the B4GALT1 product appears to be the catalytic

subunit of lactose synthase in mammary glands [75], so it appears to have played an important role in mammalian evolution. The mutation in the reported case was found to be a homozygous insertion, ins1031C [39], which leads to premature termination and loss of the C-terminal 50 amino acids [72].

Type IIe

Cells from the affected patient exhibited abnormal glycosylation of transferrin with an equal distribution of zero-, one-, two-, three-, and four-sialic acid residues [40]. In addition, there were abnormalities in glycosylation of numerous glycoproteins, and deficient sialylation of surface O-linked glycans. Transport of nucleotide sugars into the Golgi apparatus was defective, and the trafficking of proteins from the endoplasmic reticulum to the Golgi was shown to be defective. The metabolic phenotype was similar to the Chinese hamster cell mutants in the conserved oligomeric Golgi (COG) complex [86], and indirect immunofluorescence [40] indicated that the defect was in the COG7 subunit. The *COG7* gene contains 17 exons and is located on chromosome 16p [76], and the index siblings were found to have homozygous mutations in *COG7*, IVS1+4A>C [40].

Type II f

A defect in the posttranslational modification of glycoproteins was demonstrated [77]; despite a normal sialylation pattern of transferrin and other major serum glycoproteins, there was a lack of sialyl-Lewis-X, which had effects on cell-to-cell interactions. The result was recurrent infection and megakaryocyte immaturity. Mutations were demonstrated in the *SLC35A1* gene, which codes for a transporter required for entry of CMP-sialic acid into the Golgi.

Type II g

The glycosylation pattern of serum transferrin indicated a type II CDG, and there were defects in O-glycosylation as well as N-glycosylation [42]; a homozygous nonsense mutation in the *COG1* gene was shown. In two patients with a somewhat milder phenotype [43], a homozygous splice site mutation was identified in *COG1*, part of the conserved oligomeric Golgi complex which is involved in intra-Golgi transport.

Type II h

The patterns of glycosylation are abnormal, with elevations of monosialyotransferrin [44] as well as asialo-, di-, and

trisialylated forms [45], and also abnormal glycosylation of ApoC-III, indicating a defect of O-glycosylation. One patient was found to have a homozygous missense mutation [45], and another was compound for a splice-site mutation and a 5bp deletion [44] in the *COG8* gene.

Type II i

The patient's fibroblasts showed [46] delayed retrograde (Golgi-to-endoplasmic reticulum) trafficking with brefeldin-A treatment (an indicator of COG deficiency), and that finding could be reversed by expressing wildtype *COG5* cDNA in the cells. A homozygous intronic substitution leading to exon skipping and reduced expression of the *COG5* protein was identified.

Type II j

The isoelectric focusing of serum transferrin revealed a type 2 pattern. Compound heterozygosity for a missense mutation in the *COG4* gene and a large deletion in *COG4* was identified [47].

TREATMENT

Type Ib

Oral D-mannose administration has been shown to be an effective treatment of PMI deficiency. This is possible because of the ability to convert mannose to mannose 6-phosphate by hexokinases. Doses of 100–150 mg/kg three to six times per day are effective. Symptomatic improvement is expected within weeks, but it may take several months of treatment before the biochemical parameters normalize [79].

Type II c

Oral fucose supplementation was beneficial in treatment of one patient with CDG-IIc [36] at doses up to nearly 500 mg/kg per day. By contrast, no effect was observed in three of the Arab patients [38], even when using equivalent dosage [37]. It is possible that the difference relates to the observed effects of the mutations, primarily affecting the enzyme's K_m in the responsive form, as opposed to a reduction of the V_{max} in the nonresponsive form [71].

Other types

No effective treatment has been reported.

REFERENCES

- Jaeken J, Matthijs G. Congenital disorders of glycosylation. *Annu Rev Genomics Hum Genet* 2001; **2**: 129.
- Aebi M, Helenius A, Schenk B *et al*. Carbohydrate-deficient glycoprotein syndromes become congenital disorders of glycosylation: an updated nomenclature for CDG. First International Workshop on CDGS. *Glycoconj J* 1999; **16**: 669.
- Van den Steen P, Rudd PM, Dwek RA, Opdenakker G. Concepts and principles of O-linked glycosylation. *Crit Rev Biochem Mol Biol* 1998; **33**: 151.
- de Koning TJ, Dorland L, Van Diggelen OP *et al*. A novel disorder of N-glycosylation due to phosphomannose isomerase deficiency. *Biochem Biophys Res Commun* 1998; **245**: 38.
- Marquardt T, Denecke J. Congenital disorders of glycosylation: review of their molecular bases, clinical presentations and specific therapies. *Eur J Pediatr* 2003; **162**: 359.
- Jaeken J, Matthijs G, Saudubray JM *et al*. Phosphomannose isomerase deficiency: a carbohydrate-deficient glycoprotein syndrome with hepatic-intestinal presentation. *Am J Hum Genet* 1998; **62**: 1535.
- Schollen E, Dorland L, de Koning TJ *et al*. Genomic organization of the human phosphomannose isomerase (MPI) gene and mutation analysis in patients with congenital disorders of glycosylation type Ib (CDG-Ib). *Hum Mutat* 2000; **16**: 247.
- Kjaergaard S, Westphal V, Davis JA *et al*. Variable outcome and the effect of mannose in congenital disorder of glycosylation Type Ib (CDG-Ib). *J Inherit Metab Dis* 2000; **23**(Suppl. 1): 184.
- Burda P, Borsig L, Rijk-van Andel J *et al*. A novel carbohydrate-deficient glycoprotein syndrome characterized by a deficiency in glucosylation of the dolichol-linked oligosaccharide. *J Clin Invest* 1998; **102**: 647.
- Stibler H, Stephani U, Kutsch U. Carbohydrate-deficient glycoprotein syndrome – a fourth subtype. *Neuropediatrics* 1995; **26**: 235.
- Korner C, Knauer R, Stephani U *et al*. Carbohydrate deficient glycoprotein syndrome type IV: deficiency of dolichyl-P-Man: Man(5)GlcNAc(2)-PP-dolichyl mannosyltransferase. *EMBO J* 1999; **18**: 6816.
- Kim S, Westphal V, Srikrishna G *et al*. Dolichol phosphate mannose synthase (DPM1) mutations define congenital disorder of glycosylation Ie (CDG-Ie). *J Clin Invest* 2000; **105**: 191.
- Imbach T, Schenk B, Schollen E *et al*. Deficiency of dolichol-phosphate-mannose synthase-1 causes congenital disorder of glycosylation type Ie. *J Clin Invest* 2000; **105**: 233.
- Garcia-Silva MT, Matthijs G, Schollen E *et al*. Congenital disorder of glycosylation (CDG) type Ie. A new patient. *J Inherit Metab Dis* 2004; **27**: 591.
- Kranz C, Denecke J, Lehrman MA *et al*. A mutation in the human MPDU1 gene causes congenital disorder of glycosylation type If (CDG-If). *J Clin Invest* 2001; **108**: 1613.
- Schenk B, Imbach T, Frank CG *et al*. MPDU1 mutations underlie a novel human congenital disorder of glycosylation, designated type If. *J Clin Invest* 2001; **108**: 1687.
- Chantret I, Dupre T, Delenda C *et al*. Congenital disorders of glycosylation type Ig are defined by a deficiency in dolichyl-P-mannose: Man7GlcNAc2-PP-dolichyl mannosyltransferase. *J Biol Chem* 2002; **277**: 25815.
- Grubenmann CE, Frank CG, Kjaergaard S *et al*. ALG12 mannosyltransferase defect in congenital disorder of glycosylation type Ig. *Hum Mol Genet* 2002; **11**: 2331.
- Chantret I, Dancourt J, Dupre T *et al*. A deficiency in dolichyl-P-glucose: Glc1Man₃GlcNAc₂-PP-dolichyl alpha3-glucosyltransferase defines a new subtype of congenital disorders of glycosylation. *J Biol Chem* 2003; **278**: 9962.
- Schollen E, Frank CG, Keldermans L *et al*. Clinical and molecular features of three patients with congenital disorders of glycosylation type Ih (CDG-Ih) (ALG8 deficiency). *J Med Genet* 2004; **41**: 550.
- Thiel C, Schwarz M, Peng J *et al*. A new type of congenital disorder of glycosylation (CDG-Ii) provides new insights into the early steps of dolichol-linked oligosaccharide biosynthesis. *J Biol Chem* 2003; **278**: 22498.
- Wu X, Rush JS, Karaoglu D *et al*. Deficiency of UDP-GlcNAc: dolichol phosphate N-acetylglucosamine-1 phosphate transferase (DPAGT1) causes a novel congenital disorder of glycosylation Type Ij. *Hum Mutat* 2003; **22**: 144.
- Schwarz M, Thiel C, Lubbehusen J *et al*. Deficiency of GDP-Man: GlcNAc2-PP-dolichol mannosyltransferase causes congenital disorder of glycosylation type Ik. *Am J Hum Genet* 2004; **74**: 472.
- Kranz C, Denecke J, Lehle L *et al*. Congenital disorder of glycosylation type Ik (CDG-Ik): a defect of mannosyltransferase I. *Am J Hum Genet* 2004; **74**: 545.
- Frank CG, Grubenmann CE, Eyaid W *et al*. Identification and functional analysis of a defect in the human ALG9 gene: definition of congenital disorder of glycosylation type IL. *Am J Hum Genet* 2004; **75**: 146.
- Kranz C, Jungeblut C, Denecke J, *et al*. A defect in dolichol phosphate biosynthesis causes a new inherited disorder with death in early infancy. *Am J Hum Genet* 2007; **80**: 433-440.
- Haeuptle MA, Pujol FM, Neupert C, *et al*. Human RFT1 deficiency leads to a disorder of N-linked glycosylation. *Am J Hum Genet* 2008; **82**: 600-606.
- Lefeber DJ, Schonberger J, Morava E, *et al*. Deficiency of Dol-P-Man synthase subunit DPM3 bridges the congenital disorders of glycosylation with the dystroglycanopathies. *Am J Hum Genet* 2009; **85**: 76-86.
- Rind N, Schmeiser V, Thiel C, *et al*. A severe human metabolic disease caused by deficiency of the endoplasmic mannosyltransferase hALG11 leads to congenital disorder of glycosylation-Ip. *Hum Molec Genet* 2010; **19**: 1413-1424.
- Cantagrel V, Lefeber DJ, Ng BG, *et al*. SRD5A3 is required for converting polyprenol to dolichol and is mutated in a congenital glycosylation disorder. *Cell* 2010; **142**: 203-217.
- Ramaekers VT, Stibler H, Kint J, Jaeken J. A new variant of the carbohydrate deficient glycoproteins syndrome. *J Inherit Metab Dis* 1991; **14**: 385.
- Jaeken J, Schachter H, Carchon H *et al*. Carbohydrate deficient glycoprotein syndrome type II: a deficiency in Golgi localised

- N-acetyl-glucosaminyltransferase II. *Arch Dis Child* 1994; **71**: 123.
33. Schachter H, Jaeken J. Carbohydrate-deficient glycoprotein syndrome type II. *Biochim Biophys Acta* 1999; **1455**: 179.
34. De Praeter CM, Gerwig GJ, Bause E *et al*. A novel disorder caused by defective biosynthesis of N-linked oligosaccharides due to glucosidase I deficiency. *Am J Hum Genet* 2000; **66**: 1744.
35. Frydman M, Etzioni A, Eidlitz-Markus T *et al*. Rambam-Hasharon syndrome of psychomotor retardation, short stature, defective neutrophil motility, and Bombay phenotype. *Am J Med Genet* 1992; **44**: 297.
36. Marquardt T, Luhn K, Srikrishna G *et al*. Correction of leukocyte adhesion deficiency type II with oral fucose. *Blood* 1999; **94**: 3976.
37. Etzioni A, Tonetti M. Leukocyte adhesion deficiency II—from A to almost Z. *Immunol Rev* 2000; **178**: 138.
38. Etzioni A, Tonetti M. Fucose supplementation in leukocyte adhesion deficiency type II. *Blood* 2000; **95**: 3641.
39. Peters V, Penzien JM, Reiter G *et al*. Congenital disorder of glycosylation IIc (CDG-IIc) – a new entity: clinical presentation with Dandy-Walker malformation and myopathy. *Neuropediatrics* 2002; **33**: 27.
40. Wu X, Steet RA, Bohorov O *et al*. Mutation of the COG complex subunit gene COG7 causes a lethal congenital disorder. *Nature Med* 2004; **10**: 518.
41. Willig TN, Breton-Gorius J, Elbim C, *et al*. Macrothrombocytopenia with abnormal demarcation membranes in megakaryocytes and neutropenia with a complete lack of sialyl-Lewis-X antigen in leukocytes—a new syndrome? *Blood* 2001; **97**: 826–828.
42. Foulquier F, Vasile E, Schollen E, *et al*. Conserved oligomeric Golgi complex subunit 1 deficiency reveals a previously uncharacterized congenital disorder of glycosylation type II. *Proc Natl Acad Sci* 2006; **103**: 3764–3769.
43. Zeevaert R, Foulquier F, Dimitrov B, *et al*. Cerebrocostomandibular-like syndrome and a mutation in the conserved oligomeric Golgi complex, subunit 1. *Hum Molec Genet* 2009; **18**: 517–524.
44. Foulquier F, Ungar D, Reynders E, *et al*. A new inborn error of glycosylation due to a Cog8 deficiency reveals a critical role for the Cog1-Cog8 interaction in COG complex formation. *Hum Molec Genet* 2007; **16**: 717–730.
45. Kranz C, Ng BG, Sun L, *et al*. COG8 deficiency causes new congenital disorder of glycosylation type IIh. *Hum Molec Genet* 2007; **16**: 731–741.
46. Paesold-Burda, P, Maag C, Troxler H, *et al*. Deficiency in COG5 causes a moderate form of congenital disorders of glycosylation. *Hum Molec Genet* 2009; **18**: 4350–4356.
47. Reynders E, Foulquier F, Teles EL, *et al*. Golgi function and dysfunction in the first COG4-deficient CDG type II patient. *Hum Molec Genet* 2009; **18**: 3244–3256.
48. Stibler H, Jaeken J, Kristiansson B. Biochemical characteristics and diagnosis of the carbohydrate-deficient glycoprotein syndrome. *Acta Paediatr Scand* 1991; **375**: 21.
49. Harrison HH, Miller KL, Harbison MD, Slonim AE. Multiple serum protein abnormalities in carbohydrate-deficient glycoprotein syndrome: pathognomonic finding of two-dimensional electrophoresis? *Clin Chem* 1992; **38**: 1390.
50. Fang J, Peters V, Korner C, Hoffmann GF. Improvement of CDG diagnosis by combined examination of several glycoproteins. *J Inherit Metab Dis* 2004; **27**: 581.
51. Helander A, Bergstrom J, Freeze HH. Testing for congenital disorders of glycosylation by HPLC measurement of serum transferrin glycoforms. *Clin Chem* 2004; **50**: 954.
52. Jaeken J, Carchon H. Congenital disorders of glycosylation: a booming chapter of pediatrics. *Curr Opin Pediatr* 2004; **16**: 434.
53. Lacey JM, Bergen HR, Magera MJ *et al*. Rapid determination of transferrin isoforms by immunoaffinity liquid chromatography and electrospray mass spectrometry. *Clin Chem* 2001; **47**: 513.
54. Mills PB, Mills K, Mian N *et al*. Mass spectrometric analysis of glycans in elucidating the pathogenesis of CDG type IIx. *J Inherit Metab Dis* 2003; **26**: 119.
55. Stenson PD, Ball EV, Mort M *et al*. Human Gene Mutation Database (HGMD): 2003 update. *Hum Mutat* 2003; **21**: 577.
56. The Human Gene Mutation Database (HGMD). Cardiff University. Accessed 2004. Available from: <http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html>.
57. Imbach T, Grunewald S, Schenk B *et al*. Multi-allelic origin of congenital disorder of glycosylation (CDG)-Ic. *Hum Genet* 2000; **106**: 538.
58. Imbach T, Burda P, Kuhnert P *et al*. A mutation in the human ortholog of the *Saccharomyces cerevisiae* ALG6 gene causes carbohydrate-deficient glycoprotein syndrome type-Ic. *Proc Natl Acad Sci USA* 1999; **96**: 6982.
59. Schollen E, Martens K, Geuzens E, Matthijs G. DHPLC analysis as a platform for molecular diagnosis of congenital disorders of glycosylation (CDG). *Eur J Hum Genet* 2002; **10**: 643.
60. de Lonlay P, Seta N, Barrot S *et al*. A broad spectrum of clinical presentations in congenital disorders of glycosylation I: a series of 26 cases. *J Med Genet* 2001; **38**: 14.
61. Denecke J, Kranz C, Kemming D *et al*. An activated 59 cryptic splice site in the human ALG3 gene generates a premature termination codon insensitive to nonsense-mediated mRNA decay in a new case of congenital disorder of glycosylation type Id (CDG-Id). *Hum Mutat* 2004; **23**: 477.
62. Maeda Y, Tanaka S, Hino J *et al*. Human dolichol-phosphate-mannose synthase consists of three subunits, DPM1, DPM2 and DPM3. *EMBO J* 2000; **19**: 2475.
63. Grubenmann CE, Frank CG, Hulsmeier AJ *et al*. Deficiency of the first mannosylation step in the N-glycosylation pathway causes congenital disorder of glycosylation type Ii. *Hum Mol Genet* 2004; **13**: 535.
64. Baysal BE, Willett-Brozick JE, Badner JA *et al*. A mannosyltransferase gene at 11q23 is disrupted by a translocation breakpoint that co-segregates with bipolar affective disorder in a small family. *Neurogenetics* 2002; **4**: 43.
65. Tan J, Dunn J, Jaeken J, Schachter H. Mutations in the MGAT2 gene controlling complex N-glycan synthesis cause carbohydrate-deficient glycoprotein syndrome type II, an autosomal recessive disease with defective brain development. *Am J Hum Genet* 1996; **59**: 810.
66. Cormier-Daire V, Amiel J, Vuillaumier-Barrot S *et al*. Congenital disorders of glycosylation IIa cause growth retardation, mental retardation, and facial dysmorphism. *J Med Genet* 2000; **37**: 875.

67. Schachter H. The role of the GlcNAc(beta)1,2Man(alpha) moiety in mammalian development. Null mutations of the genes encoding UDP-N-acetylglucosamine: alpha-3-D-mannoside beta-1,2-N-acetylglucosaminyltransferase I and UDP-N-acetylglucosamine: alpha-D-mannoside beta-1,2-N-acetylglucosaminyltransferase I.2 cause embryonic lethality and congenital muscular dystrophy in mice and men, respectively. *Biochim Biophys Acta* 2002; **1573**: 292.
68. Lubke T, Marquardt T, von Figura K, Korner C. A new type of carbohydrate-deficient glycoprotein syndrome due to a decreased import of GDP-fucose into the golgi. *J Biol Chem* 1999; **274**: 25986.
69. Luhn K, Wild MK, Eckhardt M *et al*. The gene defective in leukocyte adhesion deficiency II encodes a putative GDP-fucose transporter. *Nat Genet* 2001; **28**: 69.
70. Lubke T, Marquardt T, Etzioni A *et al*. Complementation cloning identifies CDG-IIc, a new type of congenital disorders of glycosylation, as a GDP-fucose transporter deficiency. *Nat Genet* 2001; **28**: 73.
71. Etzioni A, Sturla L, Antonellis A *et al*. Leukocyte adhesion deficiency (LAD) type II/carbohydrate deficient glycoprotein (CDG) IIc founder effect and genotype/phenotype correlation. *Am J Med Genet* 2002; **110**: 131.
72. Hansske B, Thiel C, Lubke T *et al*. Deficiency of UDP-galactose: N-acetylglucosamine beta-1,4-galactosyltransferase I causes the congenital disorder of glycosylation type IIId. *J Clin Invest* 2002; **109**: 725.
73. Masri KA, Appert HE, Fukuda MN. Identification of the full-length coding sequence for human galactosyltransferase (beta-N-acetylglucosaminide: beta 1,4-galactosyltransferase). *Biochem Biophys Res Commun* 1988; **157**: 657.
74. Asano M, Furukawa K, Kido M *et al*. Growth retardation and early death of beta-1,4-galactosyltransferase knockout mice with augmented proliferation and abnormal differentiation of epithelial cells. *EMBO J* 1997; **16**: 1850.
75. Ramakrishnan B, Qasba PK. Crystal structure of lactose synthase reveals a large conformational change in its catalytic component, the beta1,4-galactosyltransferase-I. *J Mol Biol* 2001; **310**: 205.
76. Ungar D, Oka T, Brittle EE *et al*. Characterization of a mammalian Golgi-localized protein complex, COG, that is required for normal Golgi morphology and function. *J Cell Biol* 2002; **157**: 405.
77. Martinez-Duncker I, Dupre T, Piller V, *et al*. Genetic complementation reveals a novel human congenital disorder of glycosylation of type II, due to inactivation of the Golgi CMP-sialic acid transporter. *Blood* 2005; **105**: 2671-2676.
78. Niehues R, Hasilik M, Alton G *et al*. Carbohydrate-deficient glycoprotein syndrome type Ib. Phosphomannose isomerase deficiency and mannose therapy. *J Clin Invest* 1998; **101**: 1414.

α_1 -Antitrypsin deficiency

Introduction	803	Treatment	807
Clinical abnormalities	803	References	808
Genetics and pathogenesis	806		

MAJOR PHENOTYPIC EXPRESSION

In infancy and childhood: hepatic disease with conjugated hyperbilirubinemia, hepatocellular damage presenting in a neonatal hepatitis pattern or isolated hepatomegaly. In adulthood: emphysema of early onset. Each has deficiency of α_1 -antitrypsin.

INTRODUCTION

α_1 -Antitrypsin (AT) deficiency was discovered by Laurell and Eriksson [1] in 1963, when they reported five patients in whom the α_1 -globulin band was missing on agarose gel protein electrophoresis. Three had emphysema, and so did nine of 14 other patients with α_1 -AT deficiency [2]. The protein itself had been isolated in 1955 by Shultze and colleagues [3], who recognized its function as the major trypsin inhibitor of serum and the major component (90 percent) of the α_1 -globulin fraction [4]. It was evident early that partial deficiency was transmitted in an autosomal dominant manner, and that those with severe deficiency were homozygotes [5]. Extensive polymorphism of α_1 -AT was recognized first by Fagerhol [6] on electrophoresis on starch gels. The current method of choice is isoelectric focusing on polyacrylamide gel [7], and 75 variants have been recognized. Examination of the DNA for restriction fragment length polymorphism (RFLP) indicated even greater variation [8, 9].

A classification system has been developed [8] in which the variant proteins are designated as protease inhibitor (PI) types. More than 70 PI types are known, most of them with normal α_1 -AT. The normal variant was designated PIMM. The classic deficiency phenotype in which serum antitrypsin activity is about 15 percent of normal is PIZZ. PIMZ individuals are heterozygotes. PISS is the second most common form of deficiency.

The relationship of α_1 -AT deficiency to hepatic disease in infancy and childhood was discovered by Sharp and colleagues in 1969 [10].

The disease is a model in which an abnormal glycoprotein synthesized in the liver is not released from the hepatocyte into the circulation. The disease provides a model for the understanding of the processing of correctly and incorrectly folded glycoproteins in the endoplasmic reticulum [11]. The (SERPINA1) gene has been cloned and localized to chromosome 14q32.1 [12]. A number of deficiency mutations have been defined. The Z allele contains a G to A change in exon 5 that changes glutamic acid 342 to lysine (E342K) [13, 14].

The relationship of protease and elastin has led to understanding of the pathogenesis of emphysema, not only in this common disease, but in other nongenetic forms of emphysema. The gene has been isolated. Recombinant techniques have made abundant supplies of α_1 -AT for protein replacement therapy [15, 16], and gene replacement has been carried out in transgenic animals [17, 18]. In addition, the relationship between cigarette smoking and the occurrence of emphysema in individuals that have inherited this susceptibility [19] provides an interesting example of the interaction of genetics and environment, and the development of emphysema in non- α_1 -AT deficient smokers has provided a strong scientific argument against smoking [20].

CLINICAL ABNORMALITIES

Hepatic manifestations of α_1 -AT deficiency were first recognized by the detection of the deficiency in 14 patients with liver disease [10]. All were PIZZ homozygotes.

These patients developed specific manifestations of liver disease in the first year of life with early cholestasis that resolved by six months of life, but elevated serum levels of hepatocellular enzymes and hepatomegaly persisted. All but one developed cirrhosis. It has since become evident that most PIZZ individuals do not have severe liver disease. Some 10 percent of PIZZ infants have neonatal cholestasis [21], and about half of PIZZ infants who appear normal have abnormal serum levels of aminotransferases. The most frequent hepatic presentation is with a neonatal hepatitis syndrome [22]. These infants have conjugated hyperbilirubinemia and hepatomegaly. Vomiting may be projectile. Failure to thrive is common; some have low birth weights. Some have splenomegaly. There may be dark urine, indicating the presence of bilirubin. Bilirubinemia is a uniform indicator of cholestatic disease because indirect bilirubin is bound to albumin and not present in urine. Urinary bile acid concentration may be elevated [23].

This disorder is a major cause of the neonatal hepatitis syndrome. It has been found in 14–29 percent of such infants [24]. Bleeding may occur as a result of deficiency of vitamin K. Occasionally in such an infant there are acholic stools and the picture may simulate extrahepatic biliary atresia [25]. The diagnosis of α_1 -AT deficiency should obviate the usually demanding work-up for this disorder. In α_1 -AT deficiency the jaundice usually clears spontaneously by seven months of age [26].

Another presentation is with transient symptoms of liver disease occurring with intermittent infection or appendicitis at two years or later in a previously asymptomatic child [26]. In another group of patients, hepatomegaly has been found in childhood without a history of neonatal jaundice. Others may present first in childhood with what appears to be acute hepatitis.



Figure 105.1 JP: A seven-year-old girl with α_1 -antitrypsin deficiency who presented with hematemesis. She had been found to have hepatomegaly at two years of age and biopsy revealed cirrhosis. She had two spider telangiectases on the arms and a large healed incision over the liver, which was palpable 3 cm below the costal margin. The spleen was palpated at 8 cm. Endoscopy revealed esophageal varices.

In many patients, once the jaundice has subsided, clinical manifestations of liver disease do not recur, and ultimately serum levels of aminotransferases become normal [22]. A small number go on to develop chronic cirrhosis (Figure 105.1). Cirrhosis and early death have been reported in 2 percent of children with PIZZ and 14 percent of those with hepatic manifestations in infancy [27]. These patients may have spider telangiectases as early as two years of age. They may develop portal hypertension and esophageal varices. A number have died of this or other complications of cirrhosis during childhood or adolescence, even in infancy, and certainly after the development of cirrhosis. Ascites may be present with or without hypoalbuminemia and edema elsewhere.

We have observed a ten-month-old patient with α_1 -AT deficiency who presented with ascites that appeared on paracentesis to be chylous. Its protein content was 4.1 g/dL, and the serum albumin concentration was 3.5 g/dL. This is the so-called pseudochylous ascites in which some patients with chronic liver disease have lactescent fluid. Triglyceride content is not high, and this fluid can be distinguished by adding petroleum ether and shaking well, under which circumstances the triglyceride in true chylous fluid dissolves and the fluid becomes clear. Protein content has been used to distinguish exudative from transudative processes, but the range of protein content in liver disease is too great to permit this distinction. Coagulation factors may be reduced, and there may be clinical bleeding, especially gastrointestinal. Pruritis may be present in infancy, or may develop later. We have observed hyperammonemia and hepatic encephalopathy. Cirrhosis has also been observed in a number of adults who had a history of neonatal

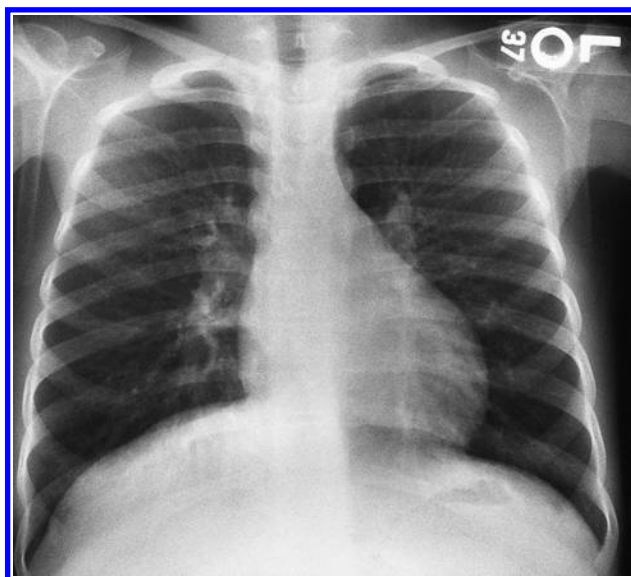


Figure 105.2 Roentgenogram of the chest of CT, a ten-year-old girl with α_1 -AT deficiency. There were no pulmonary symptoms, but bronchial markings were increased as linear densities, there was hilar prominence, and there was evidence of hyperinflation.

hepatitis or liver disease in childhood [28]. A few of these patients were found to have hepatomas on biopsy. Levels of α -fetoprotein are not usually elevated in these patients with hepatomas. Hepatocellular carcinoma has been observed.

Pulmonary disease is the most common expression of the PIZZ phenotype [29] (Figure 105.2). As many as 90 percent develop emphysema. It is classically early in onset, occurring at 20–40 years of age in smokers and 55 in nonsmokers [5, 30, 31]. It is referred to as chronic obstructive pulmonary disease or COPD. The earliest symptom is dyspnea on exertion. Cough develops in about half of the patients, and recurrent pulmonary infections are common. On examination, the patient may be thin, but the diameter of the chest is increased. Breath sounds are diminished, and the chest film reveals hyperinflation, especially in the bases. The diaphragms may be flattened. Pulmonary function tests are typical of severe emphysema consistent with a loss of pulmonary elastic recoil. Total lung capacity is impaired, as is residual volume. Air flow is limited, and diffusion capacity and maximum transpulmonary pressure are reduced. Mild hypoxemia at rest may increase with exercise. Hypocarbica and respiratory alkalosis may be associated with mild pulmonary hypertension. Electrocardiograms may show chronic strain on the right heart with right axis deviation and right atrial hypertrophy. There may be a right bundle branch block.

The early and more prominent involvement of the lower lobes [31] is in contrast to the preferential involvement of the upper lobes in acquired emphysema. Angiography reveals decreased arborization in the lower lobes in α_1 -AT deficiency [32] and radionuclide scanning shows diminished perfusion in these areas [33].

Chronic pulmonary disease in PIZZ children is not common, but some do have clinical or roentgenographic evidence of abnormality (Figure 105.2) [21, 22, 34], and more have abnormalities detectable by pulmonary function tests. Children with PI null variants may have severe emphysema early in life.

The relationship between smoking and the development of emphysema in this disease has led to the concept of genetic predisposition, in which the defective gene alone is not sufficient to produce the disease, at least by a certain age; noxious elements in the environment combine with the predisposition to yield illness. Smoking influences not only the age of onset of emphysema but the rate of its progression [35–38]. The emphysema is always associated with progressive decrease in lung function. In terms of survival, only 18 percent of PIZZ males who smoke are alive at 25 years of age, while the figure is 65 percent for nonsmokers. The comparable figures for females are 30 and 98 percent [35]. Smoking in adolescence is particularly effective, because maximal pulmonary function is not achieved [39].

The pathology of the lung has indicated that emphysema results from a destructive process involving the alveoli. Electron microscopy reveals extensive destruction of

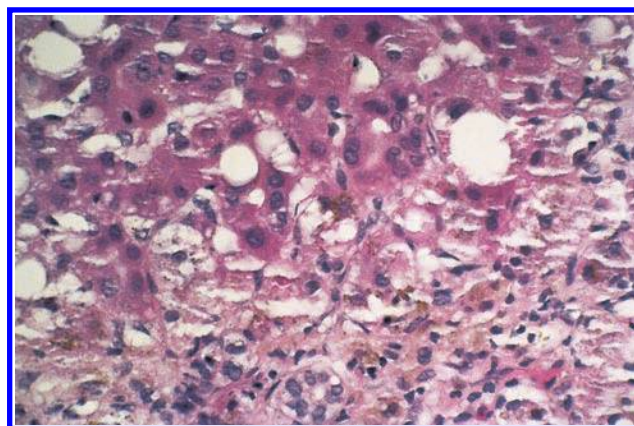


Figure 105.3 H- and E-stained biopsied liver of a patient with α_1 -AT deficiency. Hepatocytes contain eosinophilic globular inclusions. These are especially prominent in periportal areas. In addition, there is fibrosis. (Illustrations in Figures 105.3, 105.4, 105.5, 105.6, and 105.7 kindly provided by Dr Henry Krous of the Children's Hospital and Health Center, San Diego, CA.)

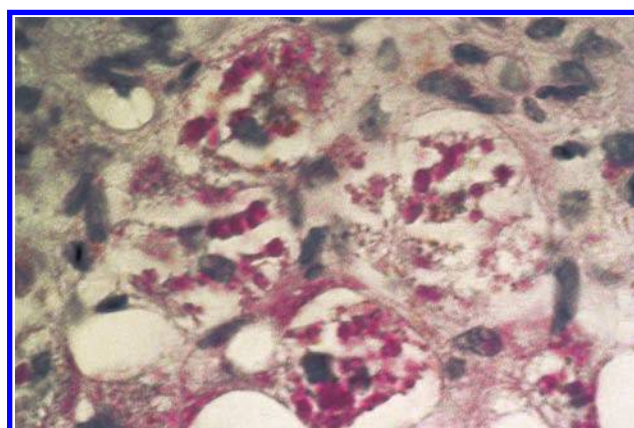


Figure 105.4 PAS stain after treatment with diastase reveals the bright pink PAS-positive inclusions.

alveolar septal walls with loss of alveolar structure and large air-filled spaces.

The pathology of the liver provided early insights into the molecular pathogenesis of the disease. The distinctive feature is the presence of globules of α_1 -AT in the cytoplasm of the hepatocytes [22, 40] (Figures 105.3 and 105.4). They are present at birth and enlarge with age. They are most prominent in the periportal regions. They stain positively with PAS stain after treatment with diastase (Figure 105.4), and positively with Oil Red O (Figure 105.5). In addition, the changes of chronic hepatic disease are nonspecific, but progressive. During the infantile cholestatic stage there is proliferation of bile ducts, fibrosis, some accumulation of fat, and occasional giant cells. Later, there is typical cirrhosis. The material has been documented to be α_1 -AT by immunofluorescence studies with antibody against α_1 -AT (Figure 105.6). In the electron microscopic picture the

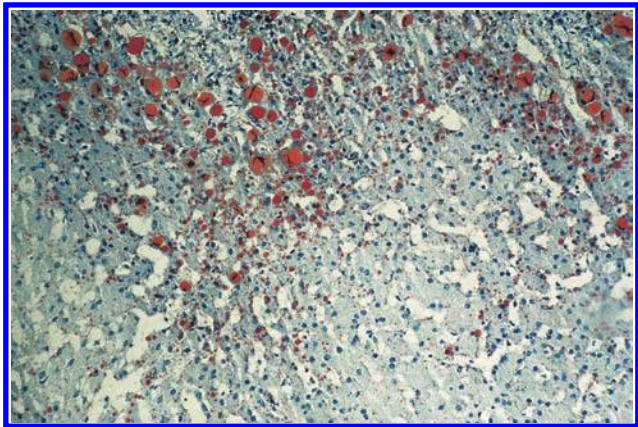


Figure 105.5 Oil Red O stain indicates some increase in fat.

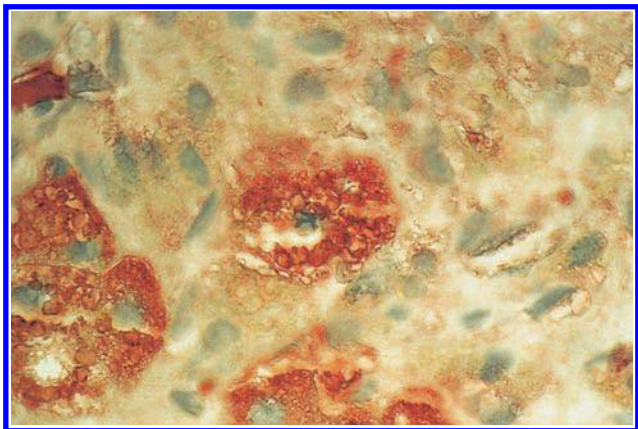


Figure 105.6 Immunoperoxidase-labeled anti- α_1 -AT antibody identifies the stored material as α_1 -AT.

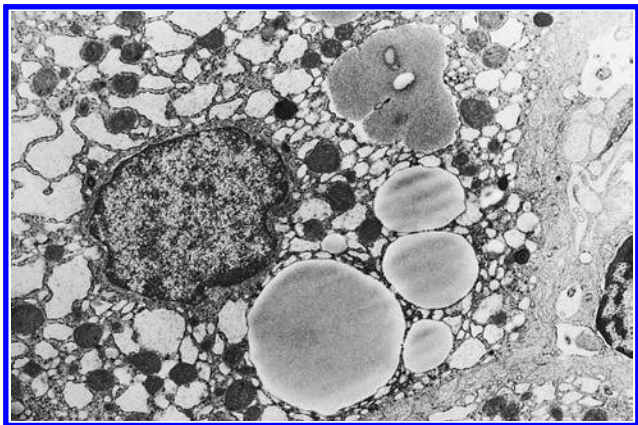


Figure 105.7 Electron microscopy shows the hepatic inclusions to be membrane-bound.

accumulated amorphous-appearing material is localized to the lumen of the endoplasmic reticulum (Figure 105.7) [22, 40]. In patients with cystic fibrosis, the Z phenotype is a risk factor for the development of severe liver disease with cirrhosis and portal hypertension [41].

In addition to disease of the lungs, membranous glomerulonephritis has been observed histopathologically, and some of these patients have had signs of renal dysfunction [42]. Some evidence of glomerular disease is common at postmortem examination in patients dying of liver disease. Immune complex disease is suggested by immunofluorescent evidence of α_1 -AT along with immunoglobulins and C3 on the glomerular basement membrane. A variety of inflammatory disorders have been associated with heterozygosity for the Z allele, including rheumatoid arthritis [43] and uveitis [44]. Severe panniculitis has been reported in 22 homozygotes [45, 46].

The diagnosis of α_1 -AT deficiency is by quantitative analysis of the content of α_1 -AT in serum. Immunologic techniques are the best. The normal range is 20–50 mmol/L, and in the Z variant it is 3–6 mmol/L. Patients with concentrations under 40 percent of normal should be PI typed.

GENETICS AND PATHOGENESIS

The normal α_1 -AT phenotype is PIMM, and the classic deficient phenotype is PIZZ [9]. M and Z are codominant autosomal alleles. The heterozygotes are PIMZ. In the PIZZ individual, serum α_1 -AT activity is 15 percent of normal. The frequency of the PIZZ phenotype in Sweden, where the disease was discovered, is approximately one in 1500. In the United States, it is one in 6000, and it is more common in those from Europe than from Africa or Asia [20]. The gene frequency for PIZZ in Sweden was reported to be 0.026 [47]. In the United States it is 0.01, while the normal M allele approximates 0.95 [25] and the S allele is 0.03. The PISS homozygote has about 60 percent of normal α_1 -AT activity. Among the many other variants [8], most have normal activity. Exceptions are PIII at 68 percent and PIPP at 30 percent of normal [48, 49] and the null variants (PI null) in which there is no detectable α_1 -AT in serum [8]. Hepatic disease of prenatal origin has been reported in a PIZ null heterozygote [50].

The α_1 -AT protein is a glycoprotein with a single polypeptide chain. Its molecular weight is 52 kDa, and its carbohydrate content of 12 percent contains a number of sialic acid residues. The protein synthesized in the liver is longer, containing a signal peptide and an N-terminal methionine [51].

The gene for α_1 -AT is located on chromosome 14 at position q32.1 [12, 52]. It is 12.2 kb in length and contains

Table 105.1 Site of the defect in classic α_1 -AT deficiency

PI phenotype	Gene	Protein	Amino acid position
M	GAG	Glutamic acid	342
Z	AAG	Lysine	342

four exons in a 1434 bp coding region [51]. In classic PIZZ α_1 -AT deficiency, a single nucleotide substitution of adenine for guanine codes for a lysine instead of glutamic acid in the M protein (Table 105.1) [13, 53]. Oligonucleotide probes have been made which recognize the Z and M sequences and can be used for diagnosis. This is particularly important for prenatal diagnosis, because prior to their development prenatal diagnosis was available only through fetal blood sampling. In the S variant a glutamic acid at position 264 is changed to valine. Null alleles represent a heterogeneous group of mutations in which a variety of different mechanisms lead to an identical phenotype [54]. Prenatal diagnosis has been carried out in two pregnancies at risk for the ZZ disease by oligonucleotide hybridization to DNA of cultured amniocytes [55]. Both fetuses were found to be MZ heterozygotes. Parental PI typing is essential for prenatal diagnosis to be sure both parents have the Z allele, and that there is no null or rare deficiency allele. Currently the method of choice is to employ PCR amplification of exon 5 and detection of the Z mutation by oligonucleotide probes labeled with 32P [56] or with biotin [57].

α_1 -AT is normally synthesized in the rough endoplasmic reticulum of hepatocytes [8]. Cultured hepatocytes secrete α_1 -AT; and *in vivo* α_1 -AT is secreted from the liver into the blood. In the Z variant, and other variants in which there is deficiency of α_1 -AT, the α_1 -AT protein is synthesized normally and levels of mRNA are normal, but the nature of the variant protein is such that it cannot be transported out of the endoplasmic reticulum. The Z variant protein isolated from the liver functions normally [58]. The failure of transport is thought to result from changes in the three-dimensional structures which interfere with normal folding and lead to local aggregation. In the normal protein, the glutamic acid at 342 is thought to form a salt bridge with lysine at 290. The substitution of the lysine at 342 would abolish this salt bridge. Support for this hypothesis was obtained by site-directed mutagenesis in which the lysine 290 in the Z protein was changed to glutamic acid, which would re-establish the salt bridge, and the resultant protein was secreted normally [59]. On the other hand, disruption of the salt bridge by changing the wild type lysine 290 to glutamic acid was followed by near normal secretion of the protein, suggesting that tertiary structure is more important than the salt bridge [60]. Aggregation of the Z protein with itself results in the aggregations that form the hepatic inclusions. There is a mobile reactive center loop in the Z protein, which locks into that of another molecule, causing dimerization [61]. This is temperature-sensitive; so an increase in body temperature with fever would be expected to increase aggregation.

The processing of α_1 -AT in the endoplasmic reticulum is aided by the transmembrane chaperone calnexin which is involved in the degradation of abnormally folded proteins [62]. Misfolded proteins are dislocated to the cytosol and degraded by the ubiquitin-proteasome system, known as endoplasmic reticulum-associated degradation (ERAD)

[11]. A null variant of α_1 -AT, Hong Kong, is a substrate for ERAD. Wild type AT was transported to the Golgi, and its carbohydrates were modified into complex glycans. In contrast, the stay in the ER of the null protein was prolonged and had protracted interaction with calnexin. Retained incompetent glycoproteins became substrates for the α -mannosidase I that tags ERAD candidates with mannose-8-glycans, which are then subject to accelerated degradation [63].

Introduction of the Z variant human gene into mice led to accumulation of the mutant human protein in mouse liver, and this was followed by hepatic necrosis and inflammation [64].

α_1 -AT in the circulation is protective of the lung because it is a very effective inhibitor of elastase and other proteolytic enzymes released from neutrophils and macrophages during the inflammatory process [65, 66]. The inactivation of elastase protects the elastic fibers of the lung [66, 67]. A growing body of evidence indicates that emphysema represents an imbalance between protease and antiprotease activity in the lung. Elastase itself produces emphysema in experimental animals as it consumes pulmonary elastin. Furthermore, cigarette smoke inactivates α_1 -AT, providing a mechanism for the next most frequent cause of emphysema [68].

TREATMENT

Treatment for hepatic disease is primarily supportive. This includes supplementation with vitamin K and vitamin D. Most patients do not go on to cirrhosis. In those that do, cholestyramine may be effective in the management of pruritis. Portacaval or splenorenal shunt may relieve esophageal varices [69]. Transplantation of the liver is curative for advanced hepatic disease [70]. The patient is then left with the prospect of pulmonary disease.

Replacement therapy has been undertaken with intravenous α_1 -AT in pharmacological amounts, and protective levels have been obtained in lung fluid as well as serum [71, 72]. The product has been licensed in the United States as an Orphan Drug. Recombinant techniques have made available ample quantities [15, 16]. Treatment requires weekly intravenous administration, and it is expensive. Analysis of data indicated that the rate of decline of forced expiratory volume was reduced, and so was mortality [38]. Trials were begun with aerosolized α_1 -AT [73], because so little intravenously administered protein reaches the lungs. Gene therapy has been accomplished in transgenic mice [17, 18].

Avoidance of smoking has been shown to make for an impressive improvement in morbidity and life expectancy [19]. This and the frequency of the gene led to a newborn screening program in Sweden in which 200,000 newborns were screened for α_1 -AT deficiency [47, 74], but the program was stopped because of unexpected psychological

effects. Parents assumed that α_1 -AT deficiency posed an immediate serious threat to the health of the child [75], and these negative feelings persisted for five to seven years [76]. The lesson is that newborn screening requires a considerable effort at public education, and this may be particularly true if the effects of the disease are long delayed. Nevertheless, screening for α_1 -AT deficiency combined with a comprehensive program aimed at the avoidance of smoking could markedly decrease morbidity.

REFERENCES

- Laurell C-B, Eriksson S. The electrophoretic alpha-1-globulin pattern of serum in alpha-1-antitrypsin deficiency. *Scand J Clin Lab Invest* 1963; **15**: 132.
- Eriksson S. Pulmonary emphysema and alpha-1-antitrypsin deficiency. *Acta Med Scand* 1964; **175**: 197.
- Schultze HE, Gollner I, Heide K *et al*. Zur Kenntnis der alpha globuline des menschlichen normalserums. *Z Naturforsch* 1955; **10**: 463.
- Schultze HE, Heide K, Haupt H. α_1 -Antitrypsin aus humanserum. *Klin Wochenschr* 1962; **40**: 427.
- Eriksson S. Studies in α_1 -antitrypsin deficiency. *Acta Med Scand* 1965; **177**(Suppl. 432): 5.
- Fagerhol MK. Serum Pi types in Norwegians. *Acta Pathol Microbiol Scand* 1967; **70**: 421.
- Allen RC, Harley RA, Talamo RC. A new method for determination of alpha-1-antitrypsin phenotypes using isoelectric focusing on polyacrylamide gel slabs. *Am J Clin Pathol* 1974; **62**: 732.
- Fagerhol MK, Cox DW. The Pi polymorphism: genetic biochemical and clinical aspects of human α_1 -antitrypsin. In: Harris H, Hirschhorn K (eds). *Advances in Human Genetics*, Vol 11. New York 1981: 1.
- Fagerhol MK, Laurell CB. The polymorphism of 'prealbumins' and α_1 -antitrypsin in human sera. *Clin Chim Acta* 1967; **16**: 199.
- Sharp HL, Bridges RA, Krivit W. Cirrhosis associated with alpha-1-antitrypsin deficiency: a previously unrecognized inherited disorder. *J Lab Clin Med* 1969; **73**: 934.
- Oda Y, Hosokawa N, Wada I, Nagata K. EDEM as an acceptor of terminally misfolded glycoproteins released from calnexin. *Science* 2003; **299**: 1394.
- Schroeder WT, Miller MF, Woo SLC, Saunders GF. Chromosomal localization of the human alpha1-antitrypsin gene (PI) to 14q31-32. *Am J Hum Genet* 1985; **37**: 868.
- Jeppsson J-O. Amino acid substitution Glu leads to Lys alpha1-antitrypsin PiZ. *FEBS Lett* 1976; **65**: 195.
- Yoshida L, Lieberman J, Gaidulis L, Ewing C. Molecular abnormality of human alpha $_1$ -antitrypsin variant (Pi-ZZ) associated with plasma activity deficiency. *Proc Natl Acad Sci USA* 1976; **73**: 1324.
- George PM, Travis J, Vissers MCM *et al*. A genetically engineered mutant of alpha1-antitrypsin protects connective tissue from neutrophil damage and may be useful in lung disease. *Lancet* 1984; **2**: 1426.
- Courtney M, Buchwalder A, Tessier L-H *et al*. High-level production of biologically active human alpha1-antitrypsin in *Escherichia coli*. *Proc Natl Acad Sci USA* 1984; **81**: 669.
- Sifers RN, Carlson JA, Clift SM *et al*. Tissue-specific expression of the human alpha-1-antitrypsin gene in transgenic mice. *Nucleic Acids Res* 1987; **15**: 1459.
- Kelsey GD, Povey S, Bygrave AE, Lovell-Badge RH. Species- and tissue-specific expression of human alpha1-antitrypsin in transgenic mice. *Genes Dev* 1987; **1**: 161.
- Buist AS. Alpha1-Antitrypsin deficiency: diagnosis treatment and control: identification of patients. *Lung* 1990; **168**(Suppl.): 543.
- Wulfsberg EA, Hoffman DE, Cohen MC. Alpha1-Antitrypsin deficiency: impact of genetic discovery on medicine and society. *JAMA* 1994; **271**: 217.
- Sveger T. Alpha1-Antitrypsin deficiency in early childhood. *Pediatrics* 1978; **62**: 22.
- Moroz SP, Cutz E, Cox DW, Sass-Kortsak A. Liver disease associated with alpha1-antitrypsin deficiency in childhood. *J Pediatr* 1976; **88**: 19.
- Karlaganis G, Nemeth A, Hammarskjold B *et al*. Urinary excretion of bile alcohols in normal children and patients with alpha1-trypsin deficiency during development of liver disease. *Eur J Clin Invest* 1982; **12**: 399.
- Cottrill K, Cook PJL, Mowat AP. Neonatal hepatitis syndrome and alpha-1-antitrypsin deficiency: an epidemiological study in south-east England. *Postgrad Med J* 1974; **50**: 376.
- Latimer JS, Sharp HL. Alpha-1-antitrypsin deficiency in childhood. *Curr Probl Pediatr* 1980; **11**: 1.
- Odièvre M, Martin J-P, Hadchouel M, Alagille D. Alpha-1-antitrypsin deficiency and liver disease in children: phenotypes manifestations and prognosis. *Pediatrics* 1976; **57**: 226.
- Sveger T. Prospective study of children with alpha1-antitrypsin deficiency; eight-year-old follow-up. *J Pediatr* 1984; **104**: 91.
- Berg NO, Eriksson S. Liver disease in adults with alpha-1-antitrypsin deficiency. *N Engl J Med* 1972; **287**: 1264.
- Eriksson S. Emphysema before and after 1963. *Ann NY Acad Sci* 1991; **624**: 1.
- Turino GM. Natural history and clinical management of emphysema in patients with and without alpha-1-antitrypsin inhibitor deficiency. *Ann NY Acad Sci* 1991; **624**: 18.
- Guenter CA, Welch MH, Russell TR *et al*. The pattern of lung disease associated with alpha1-antitrypsin deficiency. *Arch Intern Med* 1968; **122**: 254.
- Stein PD, Leu JD, Welch MH, Guenter CA. Pathophysiology of the pulmonary circulation in emphysema associated with alpha1-antitrypsin deficiency. *Circulation* 1971; **43**: 227.
- Fallat RJ, Powell MR, Kueppers F, Lilker E. 133Xe ventilatory studies in α_1 -antitrypsin deficiency. *J Nucl Med* 1972; **14**: 5.
- Talamo RC, Levison H, Lynch MJ *et al*. Symptomatic pulmonary emphysema in childhood associated with hereditary alpha-1-antitrypsin and elastase inhibitory deficiency. *J Pediatr* 1972; **79**: 20.
- Larsson C. Natural history and life expectancy in severe alpha1-antitrypsin deficiency PiZ. *Acta Med Scand* 1978; **204**: 345.

36. Thurlbeck WM, Henderson JA, Fraser RG, Bates DV. Chronic obstructive disease. A comparison between clinical roentgenologic functional and morphologic criteria in chronic bronchitis emphysema asthma and bronchiectasis. *Medicine* 1970; **49**: 81.
37. Seersholm N, Kok-Jensen A, Dirksen A. Decline in FEV1 among patients with severe hereditary α_1 -antitrypsin deficiency type PiZ. *Am J Respir Crit Care Med* 1995; **152**: 1922.
38. α_1 -Antitrypsin Deficiency Registry Study Groups: Survival and FEV1 decline in individuals with severe deficiency of alpha1-antitrypsin. *Am J Respir Crit Care Med* 1998; **158**: 49.
39. Tager IG, Munoz A, Rosner B *et al.* Effect of cigarette smoking on the pulmonary function of children and adolescents. *Am Rev Respir Dis* 1985; **131**: 752.
40. Sharp HL. Alpha-1-antitrypsin deficiency. *Hosp Pract* 1971; **55**: 83.
41. Barlett JR, Friedman KJ, Ling SC; Genetic modifiers of liver disease in cystic fibrosis. *JAMA* 2009; **302**: 1076.
42. Moroz SP, Cutz E, Balfe JW. Membranoproliferative glomerulonephritis in childhood cirrhosis associated with alpha-1-antitrypsin deficiency. *Pediatrics* 1976; **57**: 232.
43. Cox DW, Huber O. Association of severe rheumatoid arthritis with heterozygosity for α_1 -antitrypsin deficiency. *Clin Genet* 1980; **17**: 153.
44. Brewerton DA, Webley M, Murphy AH, Milford Ward AM. The α_1 -antitrypsin phenotype MZ in acute anterior uveitis. *Lancet* 1978; **1**: 1103.
45. Rubinstein HM, Jaffer AM, Kudrna JC *et al.* α_1 -Antitrypsin deficiency with severe panniculitis. *Ann Intern Med* 1977; **86**: 742.
46. Breit SN, Clark P, Robinson JP *et al.* Familial occurrence of α_1 -antitrypsin deficiency and Weber-Christian disease. *Arch Dermatol* 1983; **119**: 198.
47. Sveger T. Liver disease in alpha-1-antitrypsin deficiency detected by screening of 200 000 infants. *N Engl J Med* 1976; **294**: 1316.
48. Arnaud P, Chapius Cellier C, Vittoz P, Fudenberg H. Genetic polymorphism of serum alpha-1-protease inhibitor (alpha-1-antitrypsin): Pi I a deficient allele of the Pi system. *J Lab Clin Med* 1978; **92**: 177.
49. Fagerhol MK, Hauge HE. The PI phenotype MP. Discovery of a ninth allele belonging to the system of inherited variants of serum α_1 -antitrypsin. *Vox Sang* 1968; **15**: 396.
50. Burn J, Dunger D, Lake B. Liver damage in a neonate with alpha-1-antitrypsin deficiency due to phenotype PiZ Null (Z -). *Arch Dis Child* 1982; **57**: 311.
51. Long GI, Chandra T, Woo SLC *et al.* Complete sequence of the cDNA for human α_1 -antitrypsin and the gene for the S variant. *Biochemistry* 1984; **23**: 4828.
52. Lai EC, Kao F-T, Law ML, Woo SLC. Assignment of the α_1 -antitrypsin gene and a sequence-related gene to human chromosome 14 by molecular hybridization. *Am J Hum Genet* 1983; **35**: 385.
53. Kidd VJ, Wallace B, Itakura K, Woo SLC. α_1 -Antitrypsin deficiency detection by direct analysis of the mutation in the gene. *Nature* 1983; **304**: 230.
54. Curiel D, Brantly M, Curiel E *et al.* Alpha1-Antitrypsin deficiency caused by the alpha1-antitrypsin Null_{Mattawa} gene: An insertion mutation rendering the alpha1-antitrypsin gene incapable of producing alpha1-antitrypsin. *J Clin Invest* 1989; **83**: 1144.
55. Bruun Petersen K, Brunn Peterson G, Dahl R *et al.* α_1 Antitrypsin alleles in patients with pulmonary emphysema, detected by DNA amplifications (PCR) and oligonucleotide probes. *Eur Respir J* 1992; **5**: 531.
56. Gregersen N, Winter V, Petersen KB *et al.* Detection of point mutations in amplified single copy genes by biotin-labelled oligonucleotides: Diagnosis of variants of α_1 -antitrypsin. *Clin Chem Acta* 1989; **182**: 151.
57. Kidd VJ, Golbus MS, Wallace RB *et al.* Prenatal diagnosis of α_1 -antitrypsin deficiency by direct analysis of the mutation site in the gene. *N Engl J Med* 1984; **310**: 639.
58. Miller RR, Kuhlenschmidt MS, Coffee CJ *et al.* Comparison of the chemical physical and survival properties of normal and Z-variant α_1 -antitrypsins. *J Biol Chem* 1976; **251**: 4751.
59. Brantly M, Courtney M, Crystal RG. Repair of the secretion defect in the Z form of α_1 -antitrypsin by addition of a second mutation. *Science* 1988; **242**: 1700.
60. Foreman RC. Disruption of the Lys 290-Glu 342 salt bridge in human α_1 -antitrypsin does not prevent its synthesis and secretion. *FEBS Lett* 1987; **216**: 79.
61. Lomas DA, Evans DL, Finch JT, Carrell RW. The mechanism of Z α_1 -antitrypsin accumulation in the liver. *Nature* 1992; **357**: 605.
62. Qu D, Teckman JH, Omura S, Perlmutter DH. Degradation of a mutant secretory protein α_1 -antitrypsin Z in the endoplasmic reticulum requires proteasome activity. *J Biol Chem* 1996; **271**: 22 791.
63. Molinari M, Calanca V, Galli C *et al.* Role of EDEM in release of misfolded glycoproteins from the calnexin cycle. *Science* 2003; **299**: 1397.
64. Carlson JA, Barton Rogers B, Sifers RN *et al.* Accumulation of PiZ α_1 -antitrypsin causes liver damage in transgenic mice. *J Clin Invest* 1989; **83**: 1183.
65. Snider GL, Ciccolella DE, Morris SM *et al.* Putative role of neutrophil elastase in the pathogenesis of emphysema. *Ann NY Acad Sci* 1991; **624**: 45.
66. Gadek JE, Fells GA, Zimmerman RL *et al.* Antielastases of the human alveolar structures: implications for the protease-anti-protease theory of emphysema. *J Clin Invest* 1981; **68**: 889.
67. Gadek JE, Hunninghake GW, Fells GA *et al.* Evaluation of the protease-antiprotease theory of human destructive lung disease. *Bull Eur Physiopathol Respir* 1980; **16**: 27.
68. Gadek JE, Pacht ER. The protease-antiprotease balance within the human lung: implications for the pathogenesis of emphysema. *Lung* 1990; **168**(Suppl.): 552.
69. Sotos JF, Cutler EA, Romshe CA, Clatworthy HW Jr. Successful spleno-renal shunt and splenectomy in two patients with α_1 -antitrypsin deficiency. *J Pediatr Surg* 1981; **16**: 12.
70. Hood JM, Koep LJ, Peters RL *et al.* Liver transplantation for advanced liver disease with α_1 -antitrypsin deficiency. *N Engl J Med* 1980; **302**: 272.

71. Wewers MD, Casolaro MA, Sellers SE *et al.* Replacement therapy for alpha-1-antitrypsin deficiency associated with emphysema. *N Engl J Med* 1989; **316**: 1055.
72. Hubbard RC, Sellers S, Czerski D *et al.* Biochemical efficacy and safety of monthly augmentation therapy of alpha-1-antitrypsin deficiency. *JAMA* 1988; **260**: 1259.
73. Hubbard RC, Crystal RG. Strategies for aerosol therapy of alpha-1-antitrypsin deficiency by the aerosol route. *Lung* 1990; **168**(Suppl.): 565.
74. Laurell C-B, Sveger T. Mass screening of newborn Swedish infants for α_1 -antitrypsin deficiency. *Am J Hum Genet* 1975; **27**: 213.
75. Thelin T, McNeil TJ, Aspegren-Jansson E, Sveger T. Psychological consequences of neonatal screening for alpha-1-antitrypsin deficiency. Parental reactions to the first news of their infants' deficiency. *Acta Paediatr Scand* 1985; **74**: 787.
76. Thelin T, McNeil TF, Aspegren-Jansson E, Sveger T. Identifying children at high somatic risk: parents' long-term emotional adjustment to their children's alpha1-antitrypsin deficiency. *Acta Psychiatr Scand* 1985; **72**: 323.

Canavan disease/aspartoacylase deficiency

Introduction	811	Reproductive options	815
Clinical abnormalities	811	Treatment	816
Genetics and pathogenesis	814	References	816

MAJOR PHENOTYPIC EXPRESSION

Hypotonia, delayed development, megalenocephaly, optic atrophy, seizures, neurodegeneration, hypodense white matter, the imaging counterpart of histologic spongy degeneration, N-acetylaspartic aciduria and deficiency of aspartoacylase.

INTRODUCTION

Canavan disease (CD) is a progressive neurodegenerative leukodystrophy which is often fatal in the first ten years of life. It is generally agreed that the definitive delineation of this disorder as a distinct clinical and pathologic entity was that of van Bogaert and Bertrand in 1949 [1, 2]. Canavan's report in 1931 [3] was of an infant with a prominently enlarged head and spongy degeneration of white matter of the brain. There was a previous description by Globus and Strauss [4] in 1928. These authors and Canavan referred to these patients as having Schilder disease, but it is clear that this term has been used for a wide variety of conditions and, despite some objections [5], the term Canavan disease is the one by which this disease is known. Eiselberg [6] recognized the genetic nature of the disease. Its prevalence in Ashkenazic Jews [7–9] was recognized by van Bogaert and Bertrand [1, 2] but the disorder is panethnic.

The urinary excretion of N-acetylaspartic acid (NAA) was reported by Kvittingen, Hagenfeldt and their colleagues [10, 11]. The former reported aspartoacylase (ASP) activity (Figure 106.1) as normal [10], while the latter correctly documented the deficiency of this enzyme [11].

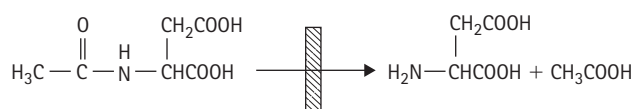


Figure 106.1 The aspartoacylase reaction.

However, neither group appreciated that they were dealing with Canavan disease. The association of aspartoacylase deficiency and N-acetylaspartic aciduria with Canavan disease was made by Matalon [12] and Divry [13, 14] and their colleagues in 1988. Aspartoacylase (EC 3.5.1.15) catalyzes the hydrolysis of N-acetylaspartic acid to aspartic and acetic acids (Figure 106.1). Kaul and his colleagues [15–18] cloned the cDNA for aspartoacylase and localized it to the terminal end of the short arm of chromosome 17. They identified a number of mutations and, in the case of the Ashkenazic Jewish population, proposed a founder effect on the basis of the predominance of two mutations [19].

CLINICAL ABNORMALITIES

Patients with this disease usually appear normal at birth and during the first few months. In the classic presentation, axial hypotonia and enlarged head are usually evident by the second to the fourth months [7–9, 20–24]. Some infants have been noted to have irritability, poor visual fixation, and poor suck from birth [25]. Spontaneous movements are decreased. The disease has been divided [19, 22] into congenital, infantile, and juvenile forms. The classic infantile form, which accounts for most of the patients, is evident by six months; the more aggressive congenital form may be evident in the first weeks of life, and the juvenile by four to five years [26–29]. There has been a suggestion of an ethnic phenotypic polymorphism. A milder clinical course, compared to the majority of CD

patients, was reported in a 21-year-old Japanese woman who was diagnosed at the age of four years. Although this patient was reported bed-ridden and had spastic quadriplegia and severely impaired mental development, she was viewed as stable [30]. The enzyme deficiency was documented in two juvenile patients [19, 29], in two of the congenital forms, and in at least 161 infantile patients [19, 31, 32]. The advent of enzyme analysis and the definition of the nature of mutation may make these classifications obsolete; correlations of phenotype with genotype may emerge.

Macrocephaly is evident by six months, and by one year the head circumference is in the 90th percentile or above [33] (Figures 106.2, 106.3, 106.4, 106.5, and 106.6). There may be delayed closure of the anterior fontanel. The weight of the brain is found to be increased at autopsy, and this is true for at least the first three years [34]; brain weight decreases in those dying later. Hypotonia, head lag or poor head control, and macrocephaly have been suggested [19] as a diagnostic triad.

With progression, milestones achieved, such as smiling and grasping may be lost. The infant becomes inattentive.



Figure 106.4 NM: Macrocephaly was prominent.



Figure 106.2 HA: A female infant with Canavan disease. Macrocephaly was already evident.



Figure 106.5 MM: A macrocephalic infant with Canavan disease.



Figure 106.3 FH: This infant with Canavan disease was more strikingly macrocephalic.



Figure 106.6 WM: This macrocephalic patient had spasticity and had assumed a tonic neck reflex posture.



Figure 106.7 MS: Also assumed a tonic reflex position. Heel cords already appeared shortened.



Figure 106.8 AG: This patient with Canavan disease demonstrated advanced spastic quadriplegia.

With time, the hypotonia is replaced by spasticity [5, 19, 35] (Figures 106.6, 106.7, and 106.8), and there may be opisthotonic posturing with tonic extensor spasms, accentuated deep tendon reflexes, and positive Babinski responses. Irritability and disturbed sleeping occur. Patients develop early blindness, and optic atrophy (Figures 106.9 and 106.10) is associated with nystagmus [9].

Seizures, tonic and clonic, develop in the second year of life in about 50 percent of patients. Weight gain falls off, and difficulty with swallowing and gastroesophageal reflux may require gastrostomy. Late findings are decerebrate or decorticate posturing.

Among patients with the juvenile form of the disease [26–29, 36–39], there may be a progressive cerebellar syndrome with dysarthria and tremor leading to spasticity and dementia. There may be optic atrophy and retinal pigmentation. Temperature instability is common, and pneumonia may be the cause of death.

Neuroimaging by computed tomography (CT) (Figure 106.11) or magnetic resonance imaging (MRI) reveals diffuse symmetrical white matter disease [35, 39–46]. T_2 weighted images on MRI show a generalized increase



Figure 106.9 A one-year-old Saudi patient with Canavan disease and macrocephaly (head circumference was 50 cm). She had central hypotonia, bilateral optic atrophy, with fisting of both hands.

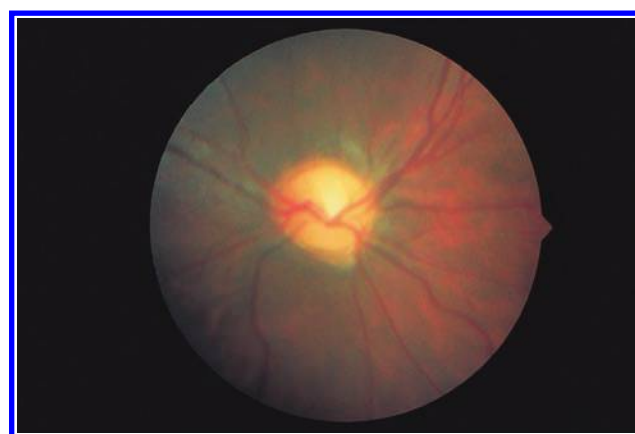


Figure 106.10 MM: Fundus photograph.

in signal throughout the subcortical cerebral white matter. The cerebellum and brainstem are less markedly involved. Decreased intensity is seen on T_1 images and CT and increased signal on T_2 . Leukodystrophy can also be demonstrated by ultrasound [47]. Changes in white matter may sometimes be absent [29]. Later, the picture is that of extreme atrophy (Figure 106.12). Honeycomb appearance of the brain in a patient with Canavan disease has been reported [48].

Nuclear magnetic resonance (NMR) spectroscopy has been used to demonstrate increased amounts of N-acetylaspartic acid in the brain *in vivo* [42–44, 49] or amounts disproportionate to choline and creatine [50].

Electroencephalograph (EEG) changes, such as

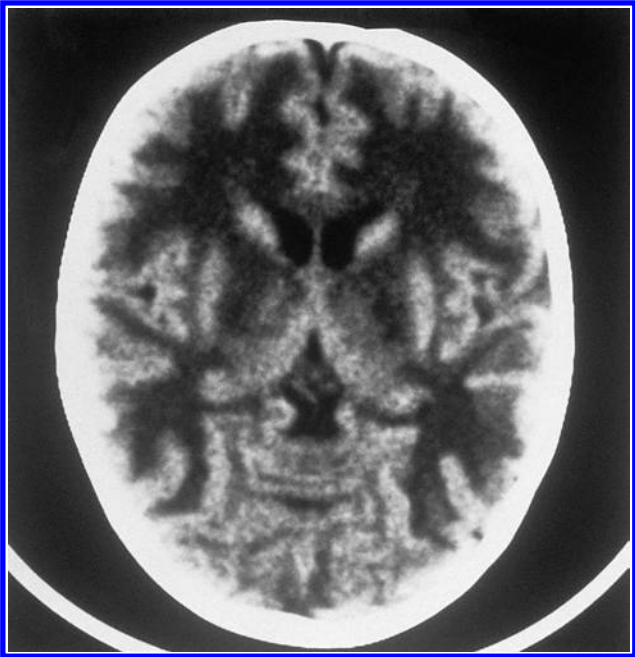


Figure 106.11 Computed tomography scan of the brain revealed diffuse symmetrical disease of the white matter.

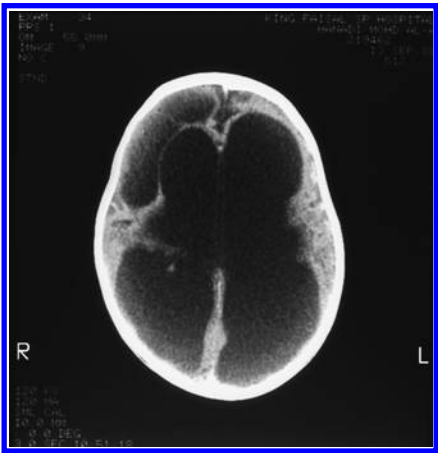


Figure 106.12 Typical white matter disease with brain filled with water as shown on computed tomography scan.

excessive slow activity and poor sleep spindle formations, have been described in approximately 40 percent of the patients [35]. The EEG may be notable for the lack of epileptiform discharges in patients who had generalized convulsions. In the majority of patients, visual or auditory evoked responses (VER and BAER) were absent, or showed delayed latencies [35].

The histopathology of Canavan disease – the spongy degeneration (Figure 106.13) [1, 2, 5, 22, 34, 51] – is the characteristic by which this disease was classically defined. The gross character of the white matter is soft or gelatinous. The vacuolization is seen in the subcortical white and lower layers of gray matter. Astrocytes are increased in

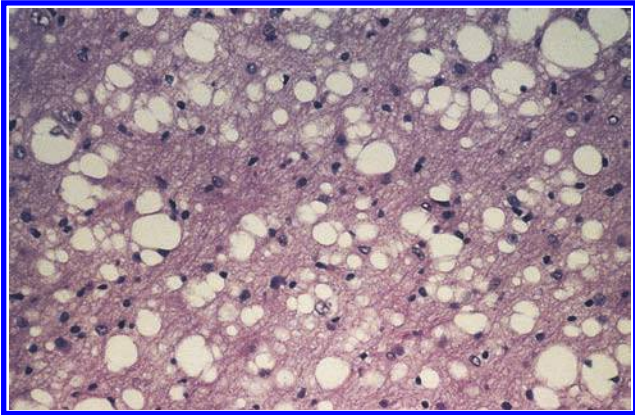


Figure 106.13 Histopathology of the brain in Canavan disease. This is the classic picture of spongy degeneration with large vacuoles.

number. Electron microscopy reveals swollen astrocytes with elongated mitochondria with distorted cristae [26, 34, 37, 51–53]. There are membrane-bound vacuoles within the swollen cytoplasm of the astrocytes. Extensive loss of myelin is mirrored in a decrease in lipid content of the white matter [54, 55].

GENETICS AND PATHOGENESIS

Canavan disease is transmitted in an autosomal recessive fashion. Consanguinity has been reported in as many as 23 percent of families [7]. The disease is panethnic in distribution, but it is particularly prevalent in Ashkenazic Jews [7, 19]; the disease is also common in Saudi Arabia [56].

The molecular defect is in the activity of aspartoacylase [12–14] (Figure 106.1). The enzyme purified from bovine brain is a 55 kDa monomer [57], and it is particularly abundant in white matter. Immunohistochemical localization of the enzyme in mammalian brain showed abundance in myelin [58]. Activity of enzyme is readily assayed in cultured fibroblasts [11, 12, 59].

The bovine cDNA was isolated by Matalon and colleagues following purification and partial sequencing of the enzyme [31]. This cDNA was employed in the isolation of the human cDNA [15]. The protein predicted for the cDNA contains 313 amino acids. The gene spans 29 kb,

Table 106.1 Some mutations in the aspartoacylase gene in Canavan disease

Nucleotide change	Protein change	Frequency	
		Jewish	Non-Jewish
854 A to G	285 Glu to Ala	0.836	0.014
693 C to A	231 Tyr to Stop	0.134	0.028
914 C to A	305 Ala to Glu	0.019	

and there are six exons; the human gene is located on chromosome 17p13-pter [18]. Two mutations were found in the Ashkenazic population [16, 60] (Table 106.1). The most frequent is a missense mutation at codon 854, which changes glutamic acid 285 to an alanine and accounts for 84 percent of 104 alleles tested [19]. Another missense mutation (Y231X) changing tyrosine to a stop-codon accounted for 13 percent of Jewish alleles [16]. A number of other mutations has been identified, including a splicing mutation in the second intron (433-2A>G) [16]. Among non-Jews, the most common mutation, accounting for 93 percent of alleles in this population, was in codon 914, and it changes alanine 305 to glutamic acid [16, 61]. A spectrum of mutations has been found in non-Jews [61]. Two novel mutations were reported in Chinese patients. They were: c.2T>C/M1T, an initiation codon mutation, and c.209A>G/N70S, which is located at the enzyme-substrate binding site [62]. In Saudi Arabia, where the disease is quite frequently seen, a novel mutation, a known large deletion, and two previously known mutations were identified [63]. Genome-wide gene expression profiling indicated that there was dysregulation of genes relevant to muscle contraction, mitochondrial oxidation, and metabolism of aspartic acid.

Carrier detection is most readily carried out by molecular techniques in families in which the mutation is known. It has been employed in the screening of Jewish populations for the two common mutations [19]. Assay of the enzyme for heterozygote detection requires cultured fibroblasts.



Figure 106.14 High voltage electrophoretic pattern of N-acetylaspargic acid.

In the presence of defective activity of aspartoacylase, the major metabolic consequence is the accumulation of NAA in body fluids. It is readily detected in the urine (Figure 106.14). The mean level in 95 patients was 1441 ± 873 mmol/mol creatinine [31]. In 53 normal individuals, the mean was 24 ± 16 mmol/mol creatinine. Levels in patients were usually 20 times the upper limit of normal. The compound is also elevated in the blood and cerebrospinal fluid [12]. The diagnosis is usually made by gas chromatography/mass spectrometry (GCMS) analysis of NAA acid in the urine [64, 65].

Increased concentrations of N-acetylasparylglutamate (NAAG) as well as NAA were associated with the myelin loss of Canavan disease [66]. Both compounds have effects on N-methyl-D-aspartate (NMDA) receptors. Oligodendrocytes express NMDA receptors, and these relationships could be relevant to myelin development or loss. However, exposure to NAAG did not lead to death of cells in the white matter. It has been hypothesized that the pathological accumulation of NAA in the extracellular fluid of the white matter leads to increase in osmotic-hydrostatic pressure which might initiate demyelination. In this view, the NAA in white matter is a product of the action of NAAG peptidase in astrocytes [67].

NAA is found in cells only in mammalian nervous tissues [68, 69], where its concentration is very high. A level of 8 mmol/g tissue has been reported [19]. This concentration is second only to glutamic acid in brain. The compound is formed from acetylCoA and aspartic acid in a reaction catalyzed by acetylCoA-L-aspartate-N-acetyl transferase [70, 71]. It is thought that the synthesis takes place in mitochondria [72]. Synthesis is in the gray matter, where aspartoacylase is undetectable [57, 73].

In studies on experimental animals, direct intracerebral ventricular injection of NAA, but not NAAG, led to biochemical evidence of oxidative stress, such as catalase activity and total radical-trapping antioxidant potential [74]. In 14-day-old rats, NAA inhibited catalase and glutathione peroxidase [75]. Treatment with liponic acid (40 mg/kg) protected against these effects [76].

In studies of aspartoacylase deficient mice there was decreased expression of neural cell markers and levels of myelin proteins. This would be consistent with effects on oligodendrocyte maturation, as well as myelination [77] building block for lipid. Hypothesizing that inability to catabolize NAA might lead to deficiency of acetate and hence acetylCoA, glyceryltriacetate was administered to rats with the tremor model of Canavan disease [78]. Improvement was noted in motor performance and myelin content, and histologic vacuolation was reduced.

REPRODUCTIVE OPTIONS

Prenatal diagnosis by assay of the enzyme in cultured amniocytes or chorionic villus material is unreliable because the activity is usually so low [79, 80]. Prenatal

diagnosis has been accomplished by GCMS analysis for NAA in amniotic fluid; isotope dilution internal standard methods are preferable [64, 81–83] because the differences between affected and unaffected values are small. Also, concentrations increase in normal amniotic fluid as gestation proceeds. A novel LC-MS/MS method for determination of NAA in amniotic fluid with minimal sample preparation has been developed for prenatal diagnosis at 16–18 weeks of gestation [84].

Analysis of the DNA is preferred if the mutation is known. The frequency of the C-854 mutation in Jewish populations makes it particularly useful [19, 82]. The mutation creates a restriction enzyme recognition sequence for *EagI* [15] converting a normal 183 bp fragment to two of 123 and 60 bp. Pre-implantation genetic diagnosis (PGD) is offered for patients with Canavan disease and other genetic disorders in Saudi Arabia, and it has been found to be very effective. To date, in 590 PGD cycles, 412 had embryo transfer with 180 pregnancies for 164 genetic diseases in Saudi Arabia.

TREATMENT

Supportive treatment is the predominant modality of treatment. Objectives are adequate nutrition and hydration, the management of or protection from infectious diseases, and protection of the airway. Contractures may be minimized via physical therapy. Programs of special education may enhance communication. Seizures are treated with antiepileptic medication. Gastrostomy may be required to maintain adequate food intake and hydration [85].

As indicated earlier, lipoic acid [76] and glyceryltriacetate [78] were therapeutic in experimental animals. Lithium citrate was reported to result in statistically significant decreased concentrations of NAA in the basal ganglia on proton magnetic resonance spectroscopy [86]. MRI suggested mild improvement in myelination. Parents reported improved alertness and social interaction. Enzyme replacement therapy has been under study [87] while recognizing that the blood–brain barrier presents a significant challenge. The human aspartoacylase was cloned, expressed, and purified and lysyl groups were modified by PEGylation yielding fully active enzyme. Administration to mice defective in this enzyme was followed by significant increase in brain enzyme activity and decrease in NAA concentrations.

REFERENCES

- van Bogaert L, Bertrand I. Sur une idiotie familiale avec dégénérescence spongieuse de néuraxe. *Acta Neurol Belg* 1949; **49**: 572.
- van Bogaert L, Bertrand I. *Spongy Degeneration of the Brain: in Infancy*. Amsterdam: North Holland Publishing Co, 1967: 3.
- Canavan MM. Schilder's encephalitis periaxialis diffusa. *Arch Neurol Psychiatr* 1931; **25**: 299.
- Globus JH, Strauss I. Progressive degenerative subcortical encephalopathy (Schilder's disease). *Arch Neurol Psychiatr* 1928; **20**: 1190.
- Banker BQ, Robertson JT, Victor M. Spongy degeneration of the central nervous system in infancy. *Neurology* 1964; **14**: 981.
- Eiselberg F. Über frühkindliche familiäre diffuse Hirnsklerose. *Z Kinderheilk* 1937; **58**: 702.
- Banker BQ, Victor H. Spongy degeneration of infancy: In: Goodman RM, Motulsky AG (eds). *Genetic Diseases Among Ashkenazi Jews*. New York: Raven Press, 1979: 201.
- Goodman RM. *Genetic Disorders Among Jewish People*. Baltimore: Johns Hopkins University Press, 1979: 109.
- Ungar M, Goodman RM. Spongy degeneration of the brain in Israel: a retrospective study. *Clin Genet* 1984; **23**: 23.
- Kvittingen EA, Guldal B, Børsting S *et al*. N-acetylaspargic aciduria in a child with a progressive cerebral atrophy. *Clin Chim Acta* 1986; **158**: 217.
- Hagenfeldt L, Bollgren I, Venizelos N. N-acetyl-aspartic aciduria due to aspartoacylase deficiency – a new aetiology of childhood leukodystrophy. *J Inherit Metab Dis* 1987; **10**: 135.
- Matalon R, Michals K, Sebesta D *et al*. Asparto-acylase deficiency and N-acetylaspargic aciduria in patients with Canavan Disease. *Am J Med Genet* 1988; **29**: 463.
- Divry P, Vianey-Liaud C, Gay C *et al*. N-acetyl-aspartic aciduria: report of three new cases in children with a neurological syndrome associated with macrocephaly and leukodystrophy. *J Inherit Metab Dis* 1988; **11**: 307.
- Divry P, Mathieu M. Aspartoacylase deficiency and N-acetylaspargic aciduria in patients with Canavan disease. *Am J Med Genet* 1989; **32**: 550.
- Kaul R, Gao GP, Balamurugan K, Matalon R. Cloning of the human aspartoacylase cDNA and a common missense mutation in Canavan disease. *Nat Genet* 1993; **5**: 118.
- Kaul R, Gao GP, Aloya M *et al*. Canavan disease: mutations among Jewish and non-Jewish patients. *Am J Hum Genet* 1994; **55**: 34.
- Kaul R, Gao GP, Michals K *et al*. A novel (cys 152 . arg) missense mutation in an Arab patient with Canavan disease. *Hum Mutat* 1995; **5**: 269.
- Kaul R, Balamurugan K, Gao GP, Matalon R. Canavan disease: genomic organization and localization of human ASPA to 17p13-ter and conservation of the ASPA gene during evolution. *Genomics* 1994; **21**: 364.
- Matalon R, Michals K, Kaul R. Canavan disease: from spongy degeneration to molecular analysis. *J Pediatr* 1995; **127**: 511.
- Buchanan DS, Davis RL. Spongy degeneration of the nervous system: a report of 4 cases with a review of the literature. *Neurology* 1965; **15**: 207.
- Sacks O, Brown WJ, Aguilar MJ. Spongy degeneration of white matter Canavan's sclerosis. *Neurology* 1965; **15**: 2165.
- Adachi M, Schneck L, Cara J, Volk BW. Spongy degeneration of the central nervous system (van Bogaert and Bertrand type; Canavan disease). *Hum Pathol* 1973; **4**: 331.

23. Gambetti P, Mellman WJ, Gonatas NK. Familial spongy degeneration of the central nervous system (van Bogaert-Bertrand disease). *Acta Neuropathol* 1969; **12**: 103.
24. Hogan GR, Richardson GP Jr. Spongy degeneration of nervous system (Canavan's disease): report of a case in an Irish-American family. *Pediatrics* 1965; **35**: 284.
25. Traeger EC, Rapin I. The clinical course of Canavan disease. *Pediatr Neurol* 1998; **18**: 207.
26. Goodhue WW Jr, Couch RD, Namiki H. Spongy degeneration of the CNS: an instance of the rare juvenile form. *Arch Neurol* 1979; **36**: 481.
27. Bruchner JM, Dom R, Robin A. Dégénérescence spongieuse juvenile du système nerveux centrale. *Rev Neurol (Paris)* 1968; **119**: 425.
28. Jellinger K, Seitelberger F. Juvenile form of spongy degeneration of the CNS. *Acta Neuropathol* 1969; **13**: 276.
29. Toft PB, Geiss-Holtorff R, Rolland MO *et al*. Magnetic resonance imaging in juvenile Canavan disease. *Eur J Pediatr* 1993; **152**: 750.
30. Mizuguchi K, Hoshino H, Hamaguchi H, Kubota M. Long term clinical course of Canavan disease – a rare Japanese case. *No To Hattatsu* 2009; **41**: 353.
31. Matalon R, Kaul R, Michals K. Canavan disease: biochemical and molecular studies. *J Inherit Metab Dis* 1993; **16**: 744.
32. Matalon R, Kaul R, Michals K. Spongy degeneration of the brain: Canavan disease. In: Duckett S (ed.). *Pediatric Neuropathology*. Baltimore: Williams and Wilkins, 1995: 625.
33. Ozand PT, Gascon GG, Dhalla M. Aspartoacylase deficiency and Canavan disease in Saudi Arabia. *Am J Med Genet* 1990; **35**: 266.
34. Adachi M, Aronson SM. Studies on spongy degeneration of the central metabolism (van Bogaert-Bertrand type). In: Aronson SM, Volk BW (eds). *Inborn Errors of Sphingolipid Metabolism*. Oxford: Pergamon Press, 1967: 129.
35. Gascon GG, Ozand PT, Mahdi A *et al*. Infantile CNS spongy degeneration – 14 cases: clinical update. *Neurology* 1990; **40**: 1876.
36. von Moers A, Sperner J, Michael T *et al*. Variable course of Canavan disease in two boys with early infantile aspartoacylase deficiency. *Dev Med Child Neurol* 1991; **33**: 824.
37. Adachi M, Volk BW. Protracted form of spongy degeneration of the central nervous system (van Bogaert and Bertrand type). *Neurology* 1968; **18**: 1084.
38. Zelnik N, Luder AS, Elpeleg ON *et al*. Protracted clinical course for patients with Canavan disease. *Dev Med Child Neurol* 1993; **35**: 355.
39. Zafeiriou DI, Kleijer WJ, Maroupoulos G *et al*. Protracted course of N-acetylaspartic aciduria in two non-Jewish siblings: identical clinical and magnetic resonance imaging findings. *Brain Dev* 1999; **21**: 205.
40. Brismar J, Brismar G, Gascon GG, Ozand P. Canavan disease CT and MR imaging of the brain. *Am J Neuroradiol* 1990; **11**: 805.
41. Matalon R, Michals J, Kaul R, Mafee M. Spongy degeneration of the brain: Canavan disease. *Int Pediatr* 1990; **5**: 121.
42. Grodd W, Krägeloh-Mann I, Petersen D *et al*. *In vivo* assessment of N-acetylaspartate in brain in spongy degeneration (Canavan disease) by proton spectroscopy. *Lancet* 1990; **336**: 437.
43. Marks HG, Caro PA, Wang ZY *et al*. Use of computed tomography magnetic resonance imaging and localized 1H magnetic resonance spectroscopy in Canavan's disease: a case report. *Ann Neurol* 1991; **30**: 106.
44. Austin SJ, Connelly A, Gadian DG *et al*. Localized 1H-NMR spectroscopy in Canavan's disease: a report of two cases. *Magn Reson Med* 1991; **19**: 439.
45. Boltshauser E, Isher W. Computerized axial tomography in spongy degeneration. *Lancet* 1976; **1**: 1123.
46. McAdams HP, Geyer CA, Done SL *et al*. CT and MR imaging of Canavan disease. *Am J Neuroradiol* 1990; **11**: 397.
47. Bühner C, Bassir C, von Moers A *et al*. Cranial ultrasound findings in aspartoacylase deficiency (Canavan disease). *Pediatr Radiol* 1993; **23**: 395.
48. Pradhan S, Goyal G. Teaching NeuroImages: Honeycomb appearance of the brain in a patient with Canavan disease. *Neurology* 2011; **76**: e68.
49. Wittsack H-J, Kugel H, Roth B, Heindel W. Quantitative measurements with localized 1H MR spectroscopy in children with Canavan's disease. *J Magn Reson Imaging* 1996; **6**: 889.
50. Barker PB, Bryan RN, Kumar AJ, Naidu S. Proton NMR spectroscopy of Canavan disease. *Neuropediatrics* 1992; **23**: 263.
51. Adachi M, Torii J, Schneck L, Volk BW. Electron microscopic and enzyme histochemical studies of the cerebellum in spongy degeneration (van Bogaert and Bertrand type). *Acta Neuropathol* 1972; **20**: 22.
52. Adornato BT, O'Brien JS, Lampert PW *et al*. Cerebral spongy degeneration of infancy: a biochemical and ultrastructural study of affected twins. *Neurology* 1972; **22**: 202.
53. Luo Y, Huang K. Spongy degeneration of the CNS in infancy. *Arch Neurol* 1984; **41**: 164.
54. Kamoshita S, Rapin I, Suzuki K, Suzuki K. Spongy degeneration of the brain. *Neurology* 1968; **19**: 975.
55. Lees MB, Folch-Pi J. A study of some human brains with pathological changes. In: Folch-Pi J (ed.). *Chemical Pathology of the Nervous System*. Oxford: Pergamon Press, 1961: 75.
56. Ozand PT, Devol EB, Gascon GG. Neuro-metabolic diseases at a national referral center: five years' experience at the King Faisal Specialist Hospital and Research Centre. *J Child Neurol* 1992; **7**(Suppl.): S4.
57. Kaul RK, Casanova J, Johnson A *et al*. Purification characterization and localization of aspartoacylase from bovine brain. *J Neurochem* 1991; **56**: 129.
58. Johnson A, Kaul R, Casanova J, Matalon R. Aspartoacylase the deficient enzyme in spongy degeneration (Canavan disease) is a myelin-associated enzyme. *J Neuropathol Exp Neurol* 1989; **48**: 349 (abstr).
59. Barash V, Flhor D, Morag B *et al*. A radiometric assay for aspartoacylase activity in fibroblasts: application for the diagnosis of Canavan's disease. *Clin Chim Acta* 1991; **201**: 175.
60. Shaag A, Anikster Y, Christensen E *et al*. The molecular basis of Canavan (aspartoacylase deficiency) disease in European non-Jewish patients. *Am J Hum Genet* 1995; **57**: 572.
61. Elpeleg ON, Shaag A. The spectrum of mutations of the aspartoacylase gene in Canavan disease in non-Jewish patients. *J Inherit Metab Dis* 1999; **22**: 531.

62. Zhang H, Liu X, Gu X. Two novel missense mutations in the aspartoacylase gene in a Chinese patient with congenital Canavan disease. *Brain Dev* 2010; **32**: 879.
63. Kaya N, Imtiaz F, Colak D *et al*. Genome-wide gene expression profiling and mutation analysis of Saudi patients with Canavan disease. *Genet Med* 2008; **10**: 675.
64. Jakobs C, ten Brink HJ, Langelaar SA *et al*. Stable isotope dilution analysis of N-acetylaspartic acid in CSF blood urine and amniotic fluid: accurate postnatal diagnosis and the potential for prenatal diagnosis of Canavan disease. *J Inherit Metab Dis* 1991; **14**: 653.
65. Kelley RI, Stamas JN. Quantification of N-acetyl-L-aspartic acid in urine by isotope dilution gas chromatography-mass spectrometry. *J Inherit Metab Dis* 1992; **15**: 97.
66. Kolodziejczyk K, Hamilton NB, Wade A *et al*. The effect of N-acetyl-aspartyl-glutamate and N-acetyl-aspartate on white matter oligodendrocytes. *Brain* 2009; **132**: 1496.
67. Baslow MH, Guilfoyle DN. Are astrocytes the missing link between lack of brain aspartoacylase activity and the spongiform leukodystrophy in Canavan disease? *Neurochem Res* 2009; **34**: 1523.
68. Tallan HH, Moore S, Stein WH. N-Acetyl-L-aspartic acid in brain. *J Biol Chem* 1956; **219**: 257.
69. Birken DL, Oldendorf WH. N-Acetylaspartic acid: a literature review of a compound prominent in 1H-NMR spectroscopic studies of brain. *Neurosci Biobehav Rev* 1989; **13**: 23.
70. Knizley H Jr. The enzymatic synthesis of N-acetyl-1-aspartic acid by a water-insoluble preparation of a cat brain acetone powder. *J Biol Chem* 1967; **242**: 4619.
71. Goldstein FB. Biosynthesis of N-acetyl-1-aspartic acid. *J Biol Chem* 1959; **234**: 2702.
72. Patel TB, Clark JB. Synthesis of N-acetyl-L-aspartate by rat brain mitochondria and its involvement in mitochondrial cytosolic carbon transport. *Biochem J* 1979; **184**: 539.
73. Truckenmiller ME, Namboodiri MAA, Brownstein MJ, Neale JH. N-Acetylation of 1-aspartate in the nervous system: differential distribution of a specific enzyme. *J Neurochem* 1985; **45**: 1658.
74. Pederzoli CD, Rockenbach FJ, Zanin FR *et al*. Intracerebroventricular administration of N-acetylaspartic acid impairs antioxidant defences and promotes protein oxidation in cerebral cortex of rats. *Metab Brain Dis* 2009; **24**: 283.
75. Pederzoli CD, Mescka CP, Magnusson AS *et al*. N-acetylaspartic acid impairs enzymatic antioxidant defenses and enhances hydrogen peroxide concentration in rat brain. *Metab Brain Dis* 2010; **25**: 251.
76. Pederzoli CD, Rosa AP, de Oliveira AS *et al*. Neuroprotective role of lipoic acid against acute toxicity of N-acetylaspartic acid. *Mol Cell Biochem* 2010; **344**: 231.
77. Mattan NS, Ghiani CA, Lloyd M *et al*. Aspartoacylase deficiency affects early postnatal development of oligodendrocytes and myelination. *Neurobiol Dis* 2010; **40**: 432.
78. Arun P, Madhavarao CN, Moffett JR *et al*. Metabolic acetate therapy improves phenotype in the tremor rat model of Canavan disease. *J Inherit Metab Dis* 2010; **33**: 195.
79. Matalon R, Michals K, Gashkoff P, Kaul R. Prenatal diagnosis of Canavan disease. *J Inherit Metab Dis* 1992; **15**: 392.
80. Bennett MJ, Gibson KM, Sherwood WG *et al*. Reliable prenatal diagnosis of Canavan disease (asparto-acylase deficiency): comparison of enzymatic and metabolite analysis. *J Inherit Metab Dis* 1993; **16**: 831.
81. Kelley RI. Prenatal diagnosis of Canavan disease by measurement of N-acetyl-aspartate in amniotic fluid. *J Inherit Metab Dis* 1993; **16**: 918.
82. Elpeleg ON, Shaag A, Anikster Y, Jakobs C. Prenatal detection of Canavan disease (aspartoacylase deficiency) by DNA analysis. *J Inherit Metab Dis* 1994; **17**: 664.
83. Ozand P T, Gascon G, Al Aqeel Al *et al*. Prenatal detection of Canavan disease. *Lancet* 1991; (i): 735.
84. Al-Dirbashi OY, Kurdi W, Imtiaz F *et al*. Reliable prenatal diagnosis of Canavan disease by measuring N-acetylaspartate in amniotic fluid using liquid chromatography tandem mass spectrometry. *Prenat Diagn* 2009; **29**: 477.
85. Matalon R, Bhatia G. Canavan Disease. In: Pagon RA, Bird TD, Dolan CR, Stephens K (eds). *GeneReviews* [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2011, updated Oct 2009.
86. Assadi M, Janson C, Wang DJ *et al*. Lithium citrate reduces excessive intra-cerebral N-acetyl aspartate in Canavan disease. *Eur J Paediatr Neurol* 2010; **14**: 354.
87. Zano S, Malik R, Szucs S *et al*. Modification of aspartoacylase for potential use in enzyme replacement therapy for the treatment of Canavan disease. *Mol Genet Metab* 2011; **102**: 176.

MAJOR PHENOTYPIC EXPRESSION

The diagram illustrates the metabolic pathways of H₂S in *E. coli*. H₂S is a central intermediate that can be produced from various sulfur-containing compounds or enter the respiratory chain. The pathways are as follows:

- Sulfur Metabolism:** Methionine is converted to homocysteine, which is then converted to cysteine. Cysteine can be converted to cystathionine, which is further converted to cysteine. Cysteine can also be converted to 2-oxobutyrate or mpyr. Cysteine is also converted to pyr. Cysteine is converted to mpyr.
- Respiratory Chain:** H₂S is oxidized to H₂O by ETC COX, which uses O₂ and reduces NADH and FADH₂. This process is coupled with the reduction of NADH and FADH₂ to NAD⁺ and FAD, respectively.
- Sulfur Cycle:** H₂S is oxidized to H₂S⁻ (S⁻), which is then converted to S⁻ (S⁻). The sulfur cycle is coupled with the reduction of GSH to GS⁻ and the oxidation of GSSG to GS⁻.
- Sulfur Metabolism (Continued):** H₂S is converted to SO₃ by the enzyme ETHE1 dioxxygenase. SO₃ is then converted to SO₄. H₂S is also converted to SO₃ by the enzyme ETHE1 dioxxygenase.
- Sulfur Metabolism (Continued):** H₂S is converted to SO₃ by the enzyme ETHE1 dioxxygenase. SO₃ is then converted to SO₄.
- Sulfur Metabolism (Continued):** H₂S is converted to SO₃ by the enzyme ETHE1 dioxxygenase. SO₃ is then converted to SO₄.

Figure 107.1 Metabolic pathways involved in ethymalonic encephalopathy. 2EAcrCoA, 2-ethyl-acrylyl-CoA; 2E3HPropCoA, 2-ethyl-3-hydroxy-propionyl-CoA; 2-KMVA, 2-ketomethylvalerate; 2-MBCoA, 2-methylbutyryl-CoA; allo, allosileucine; butCoA, butyryl-CoA; COX, cytochrome oxidase; crot-CoA, crotonyl-CoA; EMA, ethylmalonic acid; EMSemi, ethylmalonic semialdehyde; ETC, electron transport chain; PropCoA: propionyl-CoA, TigCoA: tiglyl-CoA, Enzymes are marked: (1) cystathionine β -synthase, (2) cystathionine γ -lyase, (3) mercaptopyruvate transulfurase, (4) cytochrome oxidase, (5) methylbutyryl-CoA dehydrogenase/short branched chain acyl-CoA dehydrogenase, (6) short chain acyl-CoA dehydrogenase.

INTRODUCTION

Ethylmalonic aciduria (Figure 107.1) is most commonly encountered in what has been termed ethylmalonic-adipic aciduria in less severe forms of glutaric aciduria type II, or multiple acylCoA dehydrogenase deficiency, which is due to deficiency in electron transport flavoprotein (ETF) or ETF dehydrogenase (Chapter 45) [1–3]. It is also encountered in short chain acylCoA dehydrogenase (SCAD) deficiency (Figure 107.1) [4]. These patients present with hypoketotic hypoglycemia, myopathic weakness, or cardiomyopathy, characteristics of disorders of fatty acid oxidation (Chapter 45).

A different type of disorder in which ethylmalonic aciduria is associated with a very different phenotype and normal oxidation of fatty acids was first reported in 1991 and 1994 [5–7]. It is recognized most readily by the association of encephalopathy, acrocyanosis, and petechiae. Death in infancy is also characteristic.

CLINICAL ABNORMALITIES

The most important abnormalities are those involving the central nervous system (Figures 107.2, 107.3, and 107.4). Hypotonia, head lag, and delayed development have been noted as early as three to four months [7, 8]. Developmental milestones have failed to be achieved. Generalized tonic-clonic seizures or infantile spasms begin in infancy and may be frequent, and there may be episodes of status



Figure 107.2 SP: A 19-month-old Hispanic-American girl with ethylmalonic aciduria. She was hypotonic and had delayed development. Petechial clusters visible in the forehead, cheeks, and chest came and went.



Figure 107.3 AP: A five-month-old girl with ethylmalonic aciduria. The facial appearance and the petechial lesions are illustrated. She was floppy, unresponsive, and had virtually constant infantile spasms. She had epicanthal folds, upslanting palpebral fissures, an upturned nose, and depressed nasal bridge.

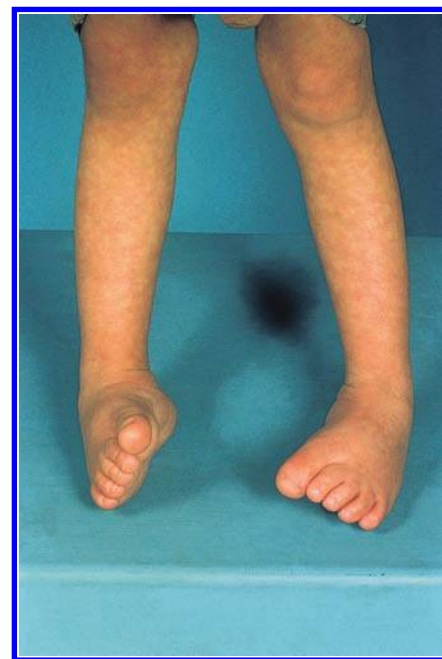


Figure 107.4 FE: A three-year-old Egyptian boy had spastic quadriplegia.

epilepticus. Deep tendon reflexes are exaggerated, and there may be ankle clonus and positive Babinski responses. One patient had microcephaly and quadriparesis (Figure 107.4) [7]. Neurological deterioration is progressive and may be rapid following intercurrent illness and leads to terminal coma and death, generally in the first to fourth year [7, 8].

Manifestations of vascular abnormality (Figures 107.2, 107.3, 107.5, 107.6, 107.7, 107.8, and 107.9) are typical in this disease [5–8], and these features are quite unique among metabolic disorders. Acrocyanosis (Figures 107.5 and 107.9) may be the mildest manifestation and it may



Figure 107.5 SP: The feet and lower legs were cold and alternately pale red or blue.



Figure 107.7 TM: A 23-month-old Yemeni girl with ethylmalonic aciduria and a large ecchymotic area on the cheek.



Figure 107.6 MM: A 12-month-old Yemeni boy with ethylmalonic aciduria. There were hemorrhagic spots on the forehead. He also had epicanthal folds.



Figure 107.8 FE: At 12 months of age, this Egyptian boy had fresh hemorrhagic streaks on his arm.

be associated with edema of the extremities. Patients also have episodic showers of petechiae, often associated with infection. One of our patients (Figure 107.2) was originally investigated for meningococemia before referral to us. There may also be ecchymoses (Figures 107.6, 107.7, and 107.9) or hemorrhagic streaks (Figures 107.9 and 107.10).

Dilated tortuous retinal vessels (Figures 107.10 and

107.11) may be seen as early as three to four months of life. Hematuria may be observed and erythrocytes were reported in the cerebrospinal fluid (CSF) [7, 9]. An association with nephrotic syndrome has been previously reported [9], and we have encountered a case with an episode of nephrosis which was responsive to conventional steroid treatment. One patient had a terminal hemoperitoneum [7]. Biopsies of the skin lesions showed nothing but hemorrhage [7]. There was no evidence for an immunologic abnormality, nor were there abnormalities of bleeding, clotting, or platelets. A markedly elevated level of plasminogen activator inhibitor-1 has been encountered [8]. Terminal events in two patients appeared to be pulmonary edema and one had cerebral edema.

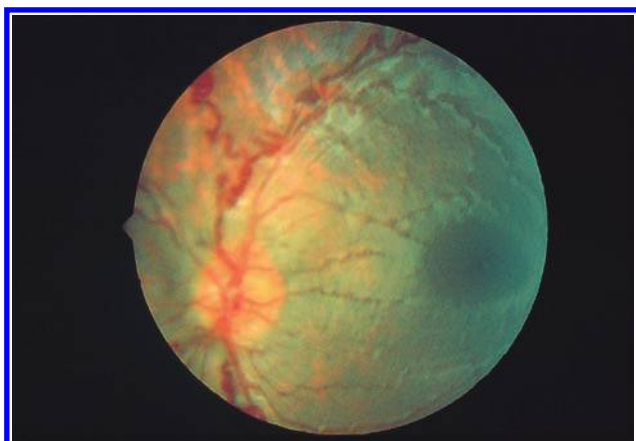


Figure 107.9 Dilated tortuous vessels in the ocular fundus of the patient in Figure 107.8.

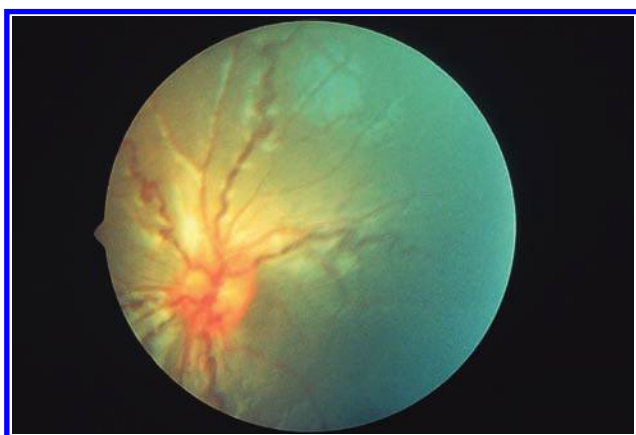


Figure 107.10 Dilated tortuous vessels in the ocular fundus of the patient in Figure 107.4.

Facial features may be mildly dysmorphic (Figures 107.6, 107.12, and 107.13) [7–10]. The facies of these patients tended to resemble each other. Some had epicanthal folds. In most, the nasal bridge was broad and depressed.

Neuroradiological findings (Figures 107.14, 107.15, and 107.16) have included frontotemporal atrophy and delayed myelination. In addition, there were areas of high T_2 intensity in the heads of the caudate nuclei, putamina, and posterior fossa [7, 8, 11]. Other abnormalities on magnetic resonance imaging (MRI) included cerebral ectopia and a tethered cord [12]. More general findings include atrophy enlargement of subarachnoid spaces [10], and occasionally hyperdensities on MRI T_2 signal of basal ganglia [10]. Electroencephalograms (EEG) were abnormal, revealing multiple focal discharges or hypsarrhythmia. Studies of nerve conduction indicated sensory neuropathy in the legs [8].

Acute metabolic crises are seen, with lactic acidemia and mild hypoglycemia. Between attacks, the blood concentrations of lactate and pyruvate remained high, and



Figure 107.11 FE: At 12 months had epicanthal folds and upward-slanting palpebral fissures.



Figure 107.12 NS: The nasal bridge was broad and she had epicanthal folds.

metabolic acidosis was compensated. Concentrations of 3.9–6.0 mmol/L of lactate and 158–230 $\mu\text{mol/L}$ of pyruvate were recorded; while in the acute attack, levels of lactate as high as 17 mmol/L were found, and there was severe acidosis with pH values of 7.05 and 7.10, and base excess of -19 without ketosis. Liver function tests may be elevated.



Figure 107.13 Patient JG at 16 months of age. Note the facial and peripheral ecchymoses and in particular the petechiae on the left arm sharply demarcated by the point of tourniquet placement.



Figure 107.14 Patient JG at 29 months of age, after three months of treatment with metronidazole and N-acetylcysteine. His cutaneous symptoms had markedly improved, as had his diarrheal stools, but he was still not bringing his hands to midline and he was displaying increasing problems with central apnea.

Creatine phosphokinase (CPK) may be normal or elevated as high as 2100 IU/L [8]. Blood ammonia is normal.

Muscle biopsy revealed increased droplets of lipid, but no ragged red fibers; on electron microscopy, there were mildly increased numbers of pleomorphic subsarcolemmal mitochondria [8]. Neuropathological examination revealed marked capillary proliferation in the substantia nigra, periaqueductal area, putamen, caudate, and medial thalamus (Figure 107.16). Endothelial cells were increased in number and size. There was a relative sparing of neurons and pallor of the background parenchyma that was quite prominent in the substantia nigra. There was vasculization of white matter tracts.

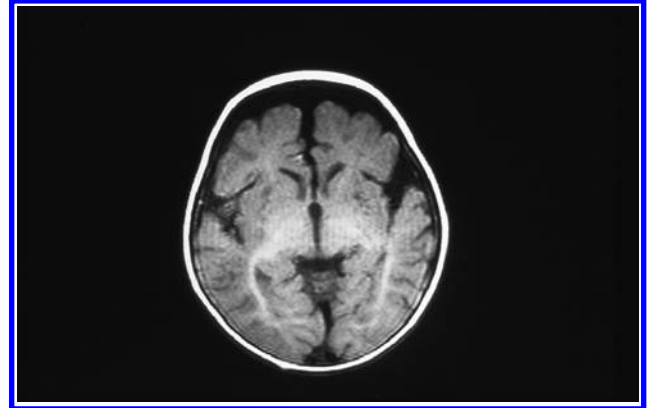


Figure 107.15 Computed tomographic scan of the brain of NS showed frontotemporal atrophy.

GENETICS AND PATHOGENESIS

The disorder is transmitted in an autosomal recessive fashion. Boys and girls have been observed in the same family. In two reports [5, 7], the parents were consanguineous. Patients reported have been Yemeni, Italian, Egyptian, Native American, and Hispanic-American.

The major metabolic abnormality is the excretion of ethylmalonic acid in the urine. Amounts reported have ranged from 54 to 2270 mmol/mol creatinine (normal <17). In some patients, the excretion of methylsuccinic acid was also elevated, but in others it was not, and levels were as high as 266 mmol/mol creatinine (normal <12) in only one patient. Adipic aciduria was not present, although in one patient a level as high as 334 mmol/mol creatinine was recorded. Tandem mass spectrometry of the blood and urine showed an elevation in C4 and C5 carnitine esters and excretion increased after treatment with carnitine. Levels of free carnitine in blood and urine are low. Acylglycine excretions have been increased, including butyrylglycine and isobutyrylglycine [6, 10], as well as 2-methylbutyrylglycine and isovalerylglycine [10].

In searching for the pathway leading to accumulation of ethylmalonic acid, elevated excretion of this compound and methylsuccinic acid after a load of isoleucine was reported by Nowaczyk *et al.* [13], and an increase in alloisoleucine was also reported. However, in our patient loading with isoleucine did not change the excretion of ethylmalonic acid or the level of alloisoleucine [8]. Nowaczyk *et al.* [13] postulated a block at the levels of 2-methylbutyrylCoA dehydrogenase; however, the activity of this enzyme was studied by Burlina *et al.* [6] and found to be normal. Furthermore, Ozand and colleagues [7] found the oxidation of ^{14}C -isoleucine to $^{14}\text{CO}_2$ to be normal in fibroblasts derived from typical patients. In these studies, the oxidation of ^{14}C -butyrate was normal, consistent with our findings on triglyceride loading. Normal oxidation of fatty acids was also observed by Burlina *et al.* [6].

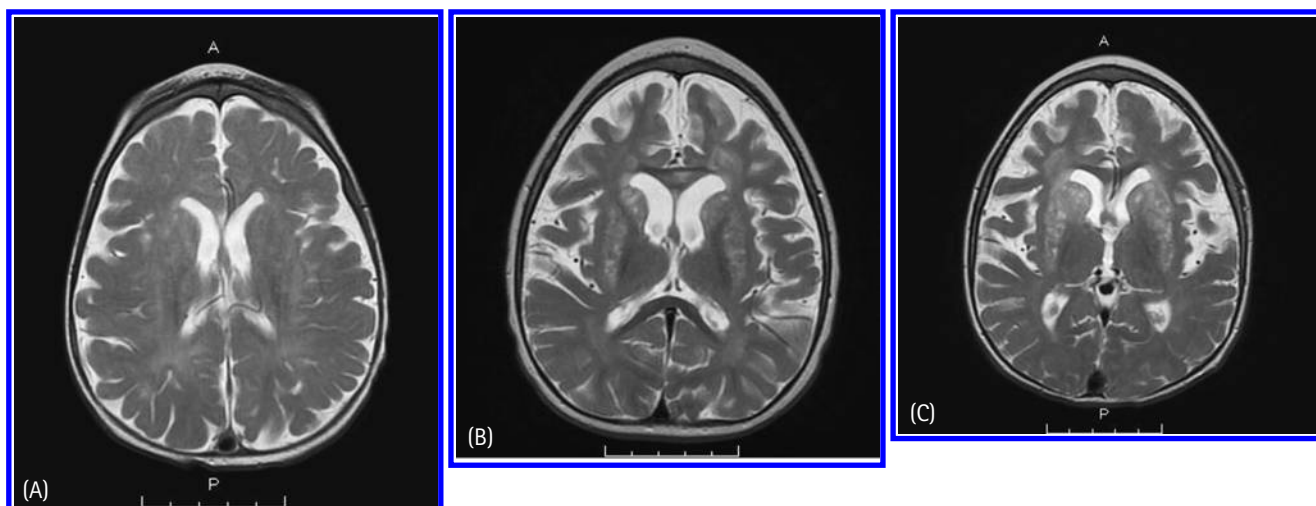


Figure 107.16 Magnetic resonance images (T_2 signal) of patient JG: (A) at six months of age, (B) at three years of age, recently following an influenza A infection, (C) at three years and two months, after two months of treatment with metronidazole and N-acetylcysteine. Note progressive atrophy and encephalomalacia of putamen, caudate, lentiform nuclei, and periventricular white matter with cystic changes. The brainstem and cerebellum appeared normal.

It is not clear why results were different following isoleucine administration in our patient and the patient of Nowaczyk *et al.* In our patient, loading with methionine was followed by an increase in excretion of ethylmalonic acid of 1.7 times to 648 mmol/mol creatinine, and that of methylsuccinic acid also rose. A relationship between this syndrome and the metabolism of sulfur amino acids was suggested by Duran and colleagues [14] who found increased excretion of inorganic thiosulfite and an absence of detectable sulfite. They also reported two sulfur-containing acidic amino acids, S-sulfocysteine and S-sulfothiocysteine, each of which can be formed nonenzymatically from thiosulfate and cysteine. The increase in excretion of ethylmalonic acid in our patient following methionine is consistent with these observations.

Methionine is converted normally to homoserine and cysteine. Homoserine is converted to 2-oxobutyric acid which could be a source of ethylmalonic acid. Cysteine is converted to 2-mercaptopyruvic acid which is metabolized to pyruvic acid and thiosulfate and ultimately sulfate [15]. Ethylmalonic acid can be formed via carboxylation of butyryl CoA (Figure 107.1) catalyzed by propionyl CoA carboxylase [16], and this appears to be the source of ethylmalonic acid found in short-chain acylCoA dehydrogenase (SCAD) deficiency and in multiple acylCoA dehydrogenase deficiency. In our patient, loading with medium-chain triglyceride did not greatly increase the excretion of ethylmalonic acid. Ethylmalonic acid could be a product of isoleucine metabolism through the R pathway after racemization of 2-oxo-3-methylvaleric acid, the precursor of alloisoleucine, from the S to the R form, which is then convertible to 2-methylbutyryl CoA, 2-ethyl-3-hydroxypropionyl CoA, and ethylmalonic semialdehyde and then to ethylmalonic acid.

Ethylhydracrylic acid is a potential source of ethylmalonic acid, but there is evidence that it is first converted to butyryl CoA [17]; furthermore, this compound was not elevated in the urine of these patients [7, 8]. It has been shown that methylsuccinic acid is formed from ethylmalonic acid in bacteria [18]. Methylsuccinic acid may also be made from 4-hydroxyisovaleric acid, which would account for its presence in glutaric aciduria type II, where isovaleryl CoA dehydrogenase is impaired. In fact, the involvement of SCAD (and short branched chain acyl-CoA dehydrogenase) had been suspected, but conventional enzyme assays have been reported to be normal [10]. The possibility that this disease is a disorder of mitochondrial electron transport had been raised [19], but studies of mitochondrial DNA in blood and muscle and the enzymes of the electron transport chain in muscle and fibroblasts have been reported to be normal [6–8, 10]. It turns out that the effects on these dehydrogenases and upon the electron transport chain are secondary.

The gene for this disease was found via homozygosity mapping to reside in chromosome 9q13 near the midpoint of the long arm [20]. Physical and functional genomic data and mutational analysis permitted identification of the gene, which has been named *ETHE1*. The protein product is targeted to the mitochondrion and actively translocated [21], where it is cleared of a 7-amino acid leader sequence [22] and internalized into the matrix. A constitutive knockout mouse model was constructed, which demonstrated impaired growth, reduced motor activity, and early death. Deficiency of cytochrome oxidase was demonstrated in several tissues, with normal activities of other electron transport chain (ETC) enzymes, including complex I and II [22]. Thiosulfate concentrations were found to be several-fold higher than control, and sulfite was

undetectable. Systemic super-physiologic concentrations of hydrogen sulfide (H_2S) were measured and shown to be sufficient to account for the observed inhibition of cytochrome oxidase (COX) and SCAD [22]. Exposure to air removes the H_2S and relieves the inhibitory effect, which presumably explains previous reports of normal SCAD and COX activities in conventional assays.

Hydrogen sulfide is a volatile molecule which has important toxic effects, but may also serve in some intracellular regulatory functions [23]. The major portion of bodily H_2S arises from bacterial metabolism, but there may also be appreciable endogenous production. Cystathionine β -synthase (using cysteine as an alternate substrate) may produce H_2S in the brain where it acts as a neuromodulator, enhancing the response of NMDA receptors [24]. H_2S is also produced in vascular endothelium through the actions of 3-mercaptopyruvate sulfurtransferase and cysteine aminotransferase [25], where it may act as a smooth muscle relaxant.

Over 30 mutations in *ETHE1* have been found in patients with ethylmalonic encephalopathy [20, 26–28]. Haplotypes for 47 patients have been tabulated [27]. There have been deletions, including heterozygous [29] and homozygous [30] deletions of exon 4. There have been at least four haplotypes in six unrelated cases which involve the mutation c.487C→T (p.R163W), as well as two other missense mutations at R163 [27], indicating a probable mutational hotspot.

TREATMENT

The disease is generally lethal in infancy or early childhood. Treatment with riboflavin, carnitine, glycine, and vitamin E have been without evident effect. Ascorbic acid and coenzyme Q10 may be used as well, but there is no formal indication of effectiveness. In our patient [8], a diet restricted in methionine led to a decrease in excretion of ethylmalonic and methylsuccinic acids and normalization of concentrations of lactic acid and bicarbonate, but the disease was relentless, and she died at 11 months of age. It is possible that systematic restriction of sulfur intake may be of some use. Upon determining the basis of the disease, Tiranti *et al.* [21] predicted that there would be a rationale to reduce exogenous H_2S production through drugs that reduce the H_2S -producing bacterial population or to reduce intrinsic H_2S production by bone marrow transplantation. Early trials of broad-spectrum enteral antibiotic (metronidazole at around 30 mg/kg per day) and an agent to increase glutathione (N-acetylcysteine at around 100 mg/kg per day) showed marked benefit in terms of the cutaneous manifestations and in some cases also in neurologic symptoms [31]. In one case we followed (Figures 107.9 and 107.17), this regimen did markedly improve cutaneous symptoms, but there was continued neurologic deterioration, central apnea, and ultimately death.

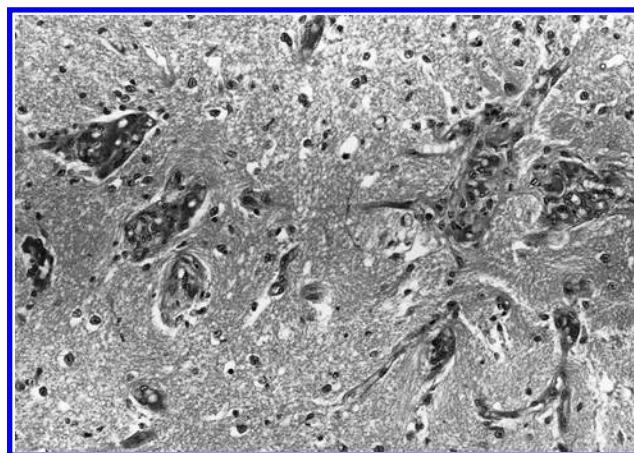


Figure 107.17 Histologic appearance of the caudate nucleus, of NP, the brother of the patient shown in Figure 107.3. He died at eight months. There was marked endothelial proliferation of capillaries and an increase in the number of capillaries (H&E, $\times 500$). (Reprinted with permission from the *Archive of Neurology* [8].)

REFERENCES

1. Nyhan WL. Ethylmalonic aciduria. In: *Abnormalities in Amino Acid Metabolism in Clinical Medicine*. Norwalk, CT: Appleton Century Crofts, 1984: 118.
2. Tanaka K, Mantago S, Genel M *et al.* New defect in fatty-acid metabolism with hypoglycaemia and organic aciduria. *Lancet* 1977; **2**: 986.
3. Goodman SI, Frerman FE, Loehr JP. Recent progress in understanding glutaric acidurias. *Enzyme* 1987; **38**: 76.
4. Bennet MJ, Gray RGF, Isherwood DM *et al.* The diagnosis and biochemical investigation of a patient with a short chain fatty acid oxidation defect. *J Inher Metab Dis* 1985; **8**: 135.
5. Burlina AB, Zachello F, Dionisi-Vici C *et al.* New clinical phenotype of branched-chain acyl-CoA defect. *Lancet* 1991; **338**: 1522.
6. Burlina AB, Dionisi-Vici C, Bennett MJ *et al.* A new syndrome with ethylmalonic aciduria and normal fatty acid oxidation in fibroblasts. *J Pediatr* 1994; **124**: 79.
7. Ozand PT, Rashed M, Millington DS *et al.* Ethylmalonic aciduria: an organic acidemia with CNS involvement and vasculopathy. *Brain Dev* 1994; **16**: 12.
8. McGowan KA, Nyhan WL, Barshop BA *et al.* The role of methionine in ethylmalonic encephalopathy with petechiae. *Arch Neurol* 2004; **61**: 570.
9. Chen E, Jerucki ER, Rinaldo P *et al.* Nephrotic syndrome and dysmorphic facial features in a new family of three affected siblings with ethylmalonic encephalopathy. *Am J Hum Genet* 1994; **55**: A2000.
10. Zafeiriou DI, Augoustides-Savvopoulou P, Haas D *et al.* Ethylmalonic encephalopathy: clinical and biochemical observations. *Neuropediatrics* 2007; **38**: 78.

11. Garcia-Silva MT, Rives A, Campos Y *et al.* Syndrome of encephalopathy petechiae and ethylmalonic aciduria. *Pediatr Neurol* 1997; **17**: 165.
12. Nowaczyk MJM, Blasser SI, Clarke JTR. Central nervous system malformations in ethylmalonic encephalopathy. *Am J Med Genet* 1998; **75**: 292.
13. Nowaczyk MJM, Lehotay DC, Platt BA *et al.* Ethylmalonic and methylsuccinic aciduria in ethylmalonic encephalopathy arise from abnormal isoleucine metabolism. *Metabolism* 1998; **47**: 836.
14. Duran M, Dorland L, van den Berg IET *et al.* The ethylmalonic acid syndrome is associated with deranged sulfur amino acid metabolism leading to urinary excretion of thiosulfate and sulfothiocysteine. Vienna VIIth International Congress of Inborn Errors of Metabolism, 1997: Abstr. 048.
15. Tanizawa K. Production of H₂S by 3-mercaptopyruvate sulphurtransferase. *J Biochem* 2011; **149**: 357–9.
16. Hegre CS, Halenz DK, Lane MD. The enzymatic carboxylation of butyryl coenzyme A. *J Am Chem Soc* 1959; **81**: 6526.
17. Baretz BH, Lollo CP, Tanaka K. Metabolism in rats *in vivo* of RS-2-methylbutyrate and N-butyrate labelled with stable isotopes at various positions: mechanism of biosynthesis and degradation of ethylmalonyl semialdehyde and ethylmalonic acid. *J Biol Chem* 1979; **254**: 34678.
18. Retey J, Smith E, Zagalak B. Investigation of the mechanism of the methylmalonyl-CoA mutase reaction with the substrate analogue: ethylmalonyl-CoA. *Eur J Biochem* 1978; **83**: 437.
19. Garavaglia B, Colamaria V, Carrara F *et al.* Muscle cytochrome-c oxidase deficiency in two Italian patients with ethylmalonic aciduria and peculiar clinical phenotype. *J Inherit Metab Dis* 1994; **17**: 301.
20. Zeviani M, Tiranti V, D'Adamo P *et al.* Ethylmalonic encephalopathy is due to mutations in ETHE1. *J Inherit Metab Dis* 2004; **27**(Suppl. 1): 177–O.
21. Tiranti V, D'Adamo P, Briem E *et al.* Ethylmalonic encephalopathy is caused by mutations in ETHE1, a gene encoding a mitochondrial matrix protein. *Am J Hum Genet* 2004; **74**: 239.
22. Tiranti V, Viscomi C, Hildebrandt T *et al.* Loss of ETHE1, a mitochondrial dioxygenase, causes fatal sulfide toxicity in ethylmalonic encephalopathy. *Nature Med* 2009; **15**: 200.
23. Kimura H. Hydrogen sulfide: from brain to gut. *Antioxid Redox Signal* 2010; **12**: 1111.
24. Abe K, Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J Neurosci* 1996; **16**: 1066.
25. Shibuya N, Mikami Y, Kimura Y *et al.* Vascular endothelium expresses 3-mercaptopyruvate sulfurtransferase and produces hydrogen sulfide. *J Biochem* 2009; **146**: 623.
26. Bischoff C, Vang S, Burlina A *et al.* High levels of EMA in suspected SCAD patients are in certain cases due to defects in the ETHE1 gene. *J Inherit Metab Dis* 2004; **27**(Suppl. 1): 176–P.
27. Mineri R, Rimoldi M, Burlina AB *et al.* Identification of new mutations in the ETHE1 gene in a cohort of 14 patients presenting with ethylmalonic encephalopathy. *J Med Genet* 2008; **45**: 473.
28. Stenson PD, Ball EV, Howells K *et al.* The Human Gene Mutation Database: providing a comprehensive central mutation database for molecular diagnostics and personalized genomics. *Hum Genom* 2009; **4**: 69.
29. Drousiotou A, DiMeo I, Mineri R *et al.* Ethylmalonic encephalopathy: application of improved biochemical and molecular diagnostic approaches. *Clin Genet* 2011; **79**: 385.
30. Ismail EA, Seoudi TM, Morsi EA, Ahmad AH. Ethylmalonic encephalopathy. Another patient from Kuwait. *Neurosciences* 2009; **14**: 78.
31. Viscomi C, Burlina AB, Dweikat I *et al.* Combined treatment with oral metronidazole and N-acetylcysteine is effective in ethylmalonic encephalopathy. *Nature Med* 2010; **16**: 869.

Disorders of creatine synthesis or transport

Introduction	827	Treatment	830
Clinical abnormalities	828	References	830
Genetics and pathogenesis	830		

MAJOR PHENOTYPIC EXPRESSION

Delayed speech and cognitive development, hypotonia, dystonia, seizures, autistic behavior, depletion of creatine in the central nervous system, increased blood and urine guanidinoacetate (in guanidinoacetatetransferase (GAMT) deficiency), low urine creatine (in arginine:glycine amidinotransferase (AGAT) deficiency), and GAMT deficiency and increased urine creatine/creatinine excretion (in creatine transporter (CRTR) deficiency).

INTRODUCTION

The disorders of creatine metabolism consist of three abnormalities in the synthesis and transport of creatine. Each disorder is characterized by severely reduced or absent creatine in the central nervous system (CNS) and neurological manifestations that range from mild developmental delay to severe neurologic disability. Our recognition of this group of disorders was initiated by Stockler and colleagues [1] in 1996 with the report of GAMT deficiency. The second disorder, AGAT deficiency, was reported in 2000 by Bianchi and associates [2]. The third disorder, CRTR, was reported by Salomons *et al.* in 2001 [3].

These disorders provide a novel approach to diagnosis in that in each the creatine peak on proton (H) magnetic resonance spectroscopy is markedly reduced or absent. In GAMT deficiency of the guanidinoacetate, peak is increased.

In the biosynthesis of creatine, the rate-limiting step is the conversion of arginine and glycine to guanidinoacetate (GAA) and ornithine, which is catalyzed by AGAT in the kidney (Figure 108.1). GAA is methylated in the liver to creatine by GAMT; S-adenosylmethionine is the methyl donor. Creatine is transported through the blood and taken up into cells against a concentration gradient by a saturable Na⁺- and Cl⁻-dependent CRTR (Figure 108.2). Creatine is converted nonenzymatically to creatinine, which is excreted in the urine in amounts approximately equal to the glomerular filtration rate [4].

Biochemical elucidation of the diagnosis is initiated by analysis of the concentrations of creatine and guanidinoacetate. In AGAT deficiency, GAA concentration in plasma is low, and creatine excretion in urine is low. In

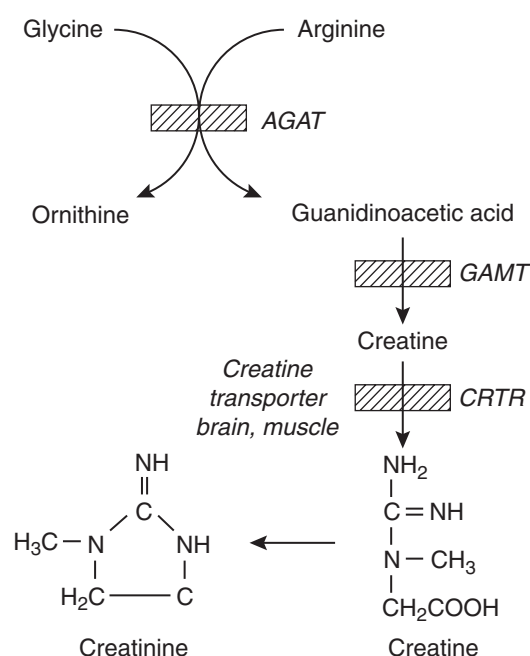


Figure 108.1 Pathways relevant to disorders of cerebral creatine deficiency. Defects are shown in arginine:glycine amidinotransferase, guanidinoacetatetransferase, and the creatine transporter.

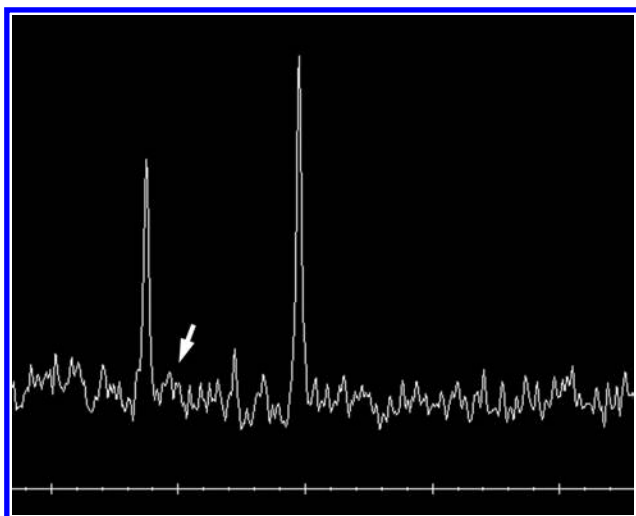


Figure 108.2 Magnetic resonance spectroscopy (MRS) of the brain of a patient with creatine transporter deficiency. Single voxel MR spectrum with TE = 144, demonstrating reduction in the creatine peak (arrow).

GAMT deficiency, concentrations of creatine are low, and GAA high. In both of these disorders patients have been recognized by organic acid analysis or amino acid analysis of the urine; since data are reported per unit creatinine excretion if creatinine is low, most compounds are reported to be high. In CRTR deficiency the excretion of creatine and creatinine are increased.

Deficiency of enzyme activity has been documented in liver in GAMT deficiency [1] and in fibroblasts in AGAT deficiency [2]. Mutations have been documented in the genes for AGAT deficiency [5] and GAMT deficiency [1, 6]. CRTR deficiency is an X-linked disorder mapped to Xq28. Uptake of creatine by cultured fibroblasts is impaired. The transporter is SLC6A8. A number and variety of mutations have been reported [7].

CLINICAL ABNORMALITIES

The syndromes of cerebral creatine deficiency have relatively recently been recognized, but it is clear that they represent an appreciable cohort of inborn errors of metabolism that interfere with CNS function. An enlarging spectrum of clinical presentation is emerging [8, 9], and these diseases are often overlooked. Certainly, patients with CRTR deficiency usually have impaired mental development and seizures. Those with GAMT deficiency may have progressive myoclonic epilepsy, but they may have regression with dystonia or hyperkinesia. They should be considered in any patient with developmental delay. The fact that some are effectively treatable [8–11] raises the stakes for early recognition.

Relative frequency is also of interest. In a study of inborn errors of metabolism in patients with unexplained

impaired mental development [11], urine samples were studied biochemically in 994 patients, and the frequency of creatine deficiency syndromes was equal to that of phenylketonuria. Similar data have been reported by others [12–15] with a frequency as high as 1 percent of patients with unexplained impaired mental development. These disorders have been considered [16] among the first approach to the algorithmic and chemical investigation of patients presenting with nonspecific impaired mental development.

The index patient with GAMT deficiency presented at 22 months with global developmental delay, and severe hypotonia; dystonic movements were noticed from the age of four to six months. By 11 months he had lost his ability to roll and was unable to sit or crawl. Seizure-like drop attacks were noted over the next few months, and an electroencephalogram (EEG) showed intermittent high-voltage slow runs with some spikes. Magnetic resonance imaging (MRI) at 12 months showed increased signal bilaterally in the globus pallidus and magnetic resonance spectroscopy (MRS) showed absent creatine and increased guanidinoacetate. GAMT activity in liver homogenate was 1.35 nmol/h. At least 37 patients have been reported with GAMT deficiency [8].

AGAT deficiency was first reported [2] in two Italian sisters aged four and six with mildly impaired mental development and severe delay in expressive speech development. One sibling had one febrile seizure. MRS studies revealed absent creatine in the CNS. Plasma creatine was normal. Plasma guanidinoacetate was slightly decreased, and urine creatinine excretion was very low. AGAT activity in cultured fibroblasts was undetectable. A third patient in this consanguineous family presented at two years of age with developmental delay, absent language, mild hypotonia, and autistic behavior with stereotypic movements of the hand. All three patients had microcephaly.

The index patient with CRTR deficiency [3] presented with hypotonia, developmental delay, and seizures. Impaired mental development was diagnosed at six years of age. His mother and maternal grandmother both had learning disabilities; the mother's sister was normal, but their brother had impaired mental development. CNS creatine was absent on MRS. Urine and plasma GAA levels were normal, but creatine levels were increased. Further studies of the three female relatives showed increased creatine in the plasma of the mother and aunt and increased urine creatine in all three women; MRS studies of the mother and aunt showed reduced, but detectable, CNS creatine. The presence of more severe symptoms in the male family members suggested X-linked inheritance, which was later confirmed by finding a mutation in the creatine transporter gene (SLC6A8) at Xq28.

In a review of 13 patients and 13 female carriers in seven unrelated families [7], the most frequent clinical findings in the families were moderate to severe mental impairment (7/7), expressive speech delay (7/7), and autistic behavior

(7/7). Seizures were common (5/7). Other less frequent symptoms included short stature and gross motor delay. Head circumference was variable. Female carrier patients have had variable degrees of learning disability (6/13). SLC6A8 deficiency was found with a prevalence of 2.1 percent in 290 patients with nonsyndromic X-linked impaired mental development [17]. Among these patients, in addition to severe hypotonia and impaired mental development, a severe movement disorder was observed with dystonia and choreoathetosis. MRS studies showed almost complete absence of creatine (Figure 108.2). An MRI showed abnormal increased signal in the peritrial white matter on the T₂-weighted images, and abnormal



Figure 108.3 Magnetic resonance imaging of the brain of a patient with creatine transporter deficiency. This sagittal T₁ image shows marked abnormal thinning of the entire corpus callosum.

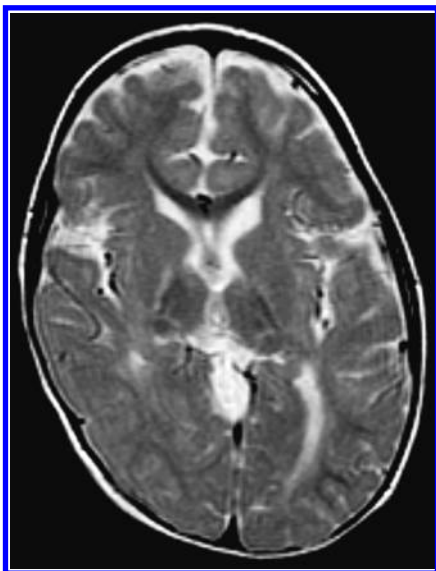


Figure 108.4 Magnetic resonance imaging of the brain. This T₂ weighted image demonstrates abnormal signal intensity in the periventricular white matter and globus pallidus bilaterally.

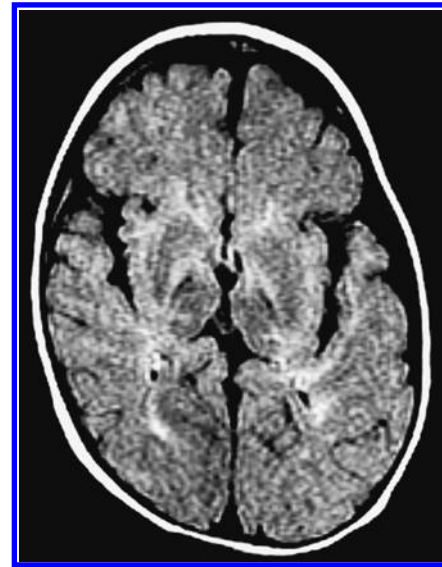


Figure 108.5 Axial FLAIR magnetic resonance imaging of the brain illustrating abnormalities in the periventricular white matter and globus pallidus.

Table 108.1 Biochemical differentiation of creatine deficiency syndromes

	GAMT Deficiency	AGAT Deficiency	CRTR Deficiency
Plasma/urine guanidinoacetate	High	Low	Normal
Urine creatine	Low	Low	High

signal intensity in bilateral globi pallidi (Figures 108.3, 108.4, and 108.5).

The creatine deficiency syndromes are all characterized chemically by depletion of cerebral creatine, which is demonstrable by MRS. Measurement of GAA in body fluids discriminates between GAMT deficiency in which it is high and AGAT deficiency in which it is low (Table 108.1). In GAMT deficiency plasma, GAA concentrations are 2–200 times elevated in urine and 10–200 times the upper limit of normal in plasma [18]. In CRTR deficiency they are normal. Creatine concentrations in blood and urine are low in AGAT and GAMT deficiencies, while the urinary creatine to creatinine ratio is elevated in CRTR deficiency.

Recently, experience has enlarged the clinical spectrum of GAMT deficiency [8], but all have presented with moderate to severe delay in development and absent or minimal speech. Regression of skills has been observed, and one patient became wheelchair bound at 14 years of age. Imaging of the brain may be normal, or there may be delayed myelination and hyperintensity of the globi pallidi. Levels of creatine are low in the cerebrospinal fluid, as well as in the plasma and urine. Seizures occurred in only two of eight recently published patients [8]; one had myoclonic epilepsy.

GENETICS AND PATHOGENESIS

The common biochemical abnormality depletion of creatine in the CNS is likely to be the primary pathogenic mechanism. Patients with GAMT and CRTR deficiencies have a movement disorder, and many have imaging abnormalities in the basal ganglia. Guanidinacetic acid is a known neurotoxin and a potential epileptogenic agent [1]. However, patients with CRTR deficiency in whom plasma GAA is normal have also presented with status epilepticus.

Creatine and phosphocreatine play an important role in intracellular energy metabolism as a high energy buffering system through the reversible reaction catalyzed by creatine kinase in which creatine and adenosine triphosphate (ATP) form phosphocreatine and adenosine diphosphate (ADP) in the mitochondria. Approximately 95 percent of creatine is found in skeletal muscle, with the rest distributed between the CNS, liver, and kidney. Some 50 percent is synthesized *de novo*, primarily in the liver, kidney, and pancreas; the rest is from dietary sources.

AGAT and GAMT deficiency are inherited as autosomal recessive disorders. Mutation analysis of the index patient with AGAT deficiency and 26 family members revealed the homozygous mutation, W149X [5].

In GAMT deficiency, the gene has been mapped to chromosome 19p13.3; four mutations were reported [1, 6]. The most frequent mutation in GAMT has been c327G>A [8], which has shown no ethnic boundaries. This is a splice site mutation which has accounted for 30 percent of abnormal alleles. The majority of mutations defined have been null mutations.

CRTR deficiency is coded for by a gene on the X chromosome Xq28 gene [18]. CRTR mutations have included missense, nonsense, and single amino acid deletions [3]. However large deletions, such as Arg514 to ter have been reported [3]. The frequency of mutations in the SLC6A8 gene has been compared to that of the fragile X mutation [17–19]. Patients with mutations in SLC6A8 have been shown to have defective uptake of creatine in cultured fibroblasts [3, 7, 20].

Enzyme activity in AGAT deficiency has been found to be undetectable in fibroblasts derived from patients [2, 5]. In GMT deficiency, GAMT enzyme activity in the index patient was 1.35 nmol/h in biopsied liver [1].

TREATMENT

Treatment with supplemental creatine, a dose of 400 mg/kg per day, was employed and resulted in significant improvement in patients with AGAT and GAMT deficiencies. In three patients with AGAT there was rapid improvement in fine-motor skills and in behavior disorder; these improvements paralleled the increase in brain levels of creatine. Speech and cognition remained significantly impaired. In GAMT deficiency, oral supplementation in the first three reported patients, with creatine monohydrate

in doses of 0.35–2.0 g/kg per day, resulted in gradual increase in the CNS creatine signal on MRS, but it was still significantly below normal. GAA levels remained high, suggesting the possibility that the compound may inhibit the transport of creatine into the CNS. The index patient did show significant clinical improvement; the movement disorder, swallowing difficulties, and seizure disorder resolved. His gross motor function improved, and he was able to walk at the age of five years. He continued to have some autistic and self-injurious behavior. Another patient showed improved psychomotor function and resolution of the seizures and globus pallidus lesions. A third showed no improvement; seizures continued. In one patient, dietary arginine restriction and ornithine supplementation (100 mg/kg per day) with an arginine-free essential amino acid formula (0.4 g/kg per day), resulted in significant reduction of plasma GAA and improvement in seizure activity and EEG [21]. Current treatment with 400–800 mg/kg of creatine and ornithine and restriction of arginine intake has led regularly to improvement [8]. Most patients have reached a plateau, but in our two patients therapeutic result has been rewarding. Presymptomatic treatment of another neonate has resulted in normal development [22], which appears to be the case in our second sibling. Supplementation of creatinine in doses as high as 750 mg/kg per day did not result in improvement in clinical manifestations or MRS creatine signal in patients with CRTR deficiency.

REFERENCES

1. Stockler S, Isbrandt D, Hanefeld F *et al*. Guanidinoacetate methyltransferase deficiency: the first inborn error of creatine metabolism in man. *Am J Hum Gene* 1996; **58**: 914.
2. Bianchi MC, Tosetti M, Fornai F *et al*. Reversible brain creatine deficiency in two sisters with normal blood creatine level. *Ann Neurol* 2000; **47**: 511.
3. Salomons GS, van Dooren SJ, Verhoeven NM *et al*. X-linked creatine-transporter gene (SLC6A8) defect: a new creatine-deficiency syndrome. *Am J Hum Genet* 2001; **68**: 1497.
4. Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. *Physiol Rev* 2000; **80**: 1107.
5. Battini R, Leuzzi V, Carducci C *et al*. Creatine depletion in a new case with AGAT deficiency: clinical and genetic study in a large pedigree. *Mol Genet Metab* 2002; **77**: 326.
6. Carducci C, Leuzzi V, Carducci C *et al*. Two new severe mutations causing guanidinoacetate methyltransferase deficiency. *Mol Genet Metab* 2000; **71**: 633.
7. Salomons GS, van Dooren SJ, Verhoeven NM *et al*. X-linked creatine transporter defect: an overview. *J Inher Metab Dis* 2003; **26**: 309.
8. Dhar SU, Scaglia F, Li F-Y *et al*. Expanded clinical and molecular spectrum of guanidinoacetate methyltransferase (GAMT) deficiency. *Mol Genet Metab* 2009; **96**: 38.

9. Stromberger C, Bodamer OA, Stockler-Ipsiroglu S. Clinical characteristics and diagnostic clues in inborn errors of creatine metabolism. *J Inherit Metab Dis* 2003; **26**: 299.
10. Stockler S, Holzbach U, Hanefeld F *et al*. Creatine deficiency in the brain: A new treatable inborn error of metabolism. *Pediatr Res* 1994; **36**: 409.
11. Sempere A, Arias A, Farre G. Study of inborn errors of metabolism in urine from patients with unexplained mental retardation. *J Inherit Metab Dis* 2010; **33**: 1–7.
12. Arias A, C. Corbella M, Fons C *et al*. Creatine transporter deficiency: prevalence among patients with mental retardation and pitfalls in metabolite screening. *Clin Biochem* 2007; **40**: 1328.
13. Clark AJ, Rosenberg EH, Almeida LS *et al*. X-linked creatine transporter (SLC6A8) mutations in about 1% of males with mental retardation of unknown etiology. *Hum Genet* 2006; **119**: 604.
14. Lion-Francois L, Cheillan D, Pitelet G *et al*. High frequency of creatine deficiency syndromes in patients with unexplained mental retardation. *Neurology* 2006; **67**: 1713.
15. Newmeyer A, Cecil KM, Schapiro M *et al*. Incidence of brain creatine transporter deficiency in males with developmental delay referred for brain magnetic resonance imaging. *J Dev Behav Pediatr* 2005; **26**: 276.
16. Garcia-Cazorla A, Wolf NL, Serrano M *et al*. Mental retardation and inborn errors of metabolism. *J Inherit Metab Dis* 2009; **32**: 597.
17. Rosenberg EH, Almeida LS, Kleefstra T *et al*. High prevalence of SLC6A8 deficiency in X-linked mental retardation. *Am J Hum Genet* 2004; **75**: 97.
18. Gregor P, Nash SR, Caron MG. Assignment of the creatine transporter gene (SLC6A8) to human chromosome Xq28 telomeric to G6PD. *Genomics* 1995; **25**: 332.
19. Hahn KA, Salomons GS, Tackels-Horne D *et al*. X-linked mental retardation with seizures and carrier manifestations is caused by a mutation in the creatine-transporter gene (SLC6A8) located in Xq28. *Am J Hum Genet* 2002; **70**: 1349.
20. Verhoeven NM, Schor DSM, Roos B *et al*. Stable-isotope dilution enzyme assays for the detection of inborn errors of creatine synthesis. *J Inherit Metab Dis* 2001; **24**: 118.
21. Schulze A, Ebinger F, Rating D, Mayatepek E. Improving treatment of guanidinoacetate methyltransferase deficiency: reduction of guanidinoacetic acid in body fluids by arginine restriction and ornithine supplementation. *Mol Genet Metab* 2001; **74**: 413.
22. Schulze A, Hoffmann GF, Bachert P *et al*. Presymptomatic treatment of neonatal guanidinoacetate methyltransferase deficiency. *Neurology* 2006; **67**: 719.

Appendix: Differential diagnosis of clinical phenotypes

CONTENTS

Acidosis, hyperchloremic	835	Hepatic failure – acute	839
Alopecia	835	Hydrops fetalis	840
Angiokeratomas	835	3-Hydroxyglutaric aciduria	840
Apparent acute encephalitis	835	Hyperammonemia	840
Arthritis	835	Hypercalcemia	840
Black pigment deposition	835	Hypertyrosinemia	840
Bleeding tendency	835	Hypoketotic hypoglycemia	840
Calcification of basal ganglia	835	Hypophosphatemia	841
Cardiomyopathy	835	Hypouricemia	841
Carnitine esters	836	Ichthyosis	841
Cataracts – lenticular opacity	836	Ichthyosis and retinal disease	841
Cerebral calcification	836	Inverted nipples	841
Cerebral vascular disease	836	Isolated deficiency of speech as presentation in metabolic disease	841
Cerebrospinal fluid lymphocytosis	836	Lactic acidemia	841
Cerebrospinal fluid protein elevation	836	Leigh syndrome	841
Cherry red macular spots	837	Leukopenia with or without thrombopenia and anemia	841
Cholestatic jaundice	837	Macrocephaly	841
Chondrodysplasia phenotypes	837	Megaloblastic anemia	842
Chronic pancreatitis	837	Metabolic acidosis and ketosis	842
Cirrhosis of the liver	837	Methylmalonic aciduria	842
Corneal opacity	837	Mongolian spot – extensive	842
Corpus callosum agenesis	837	Myocardial infarction-cerebral vascular disease	842
Creatine kinase – elevated	837	Neonatal hepatic presentations in metabolic diseases	842
Dermatosis	838	Neutropenia	842
Diabetes mellitus – erroneous diagnosis	838	Odd or unusual odor	842
Diarrhea	838	Optic atrophy	843
Disorders of folate metabolism or transport	838	Orotic aciduria	843
Drugs or other triggers of symptoms in metabolic diseases	838	Osteoporosis and fractures	843
Dysostosis multiplex	838	Pain and elevated erythrocyte sedimentation rate	843
Ectopia lentis (dislocation of the lens)	838	Pancreatitis	843
EEG burst suppression pattern	838	Paralysis of upward gaze	843
Exercise intolerance	838	Photophobia	843
Failure to thrive	839	Polycystic kidneys	843
Fever syndromes	839	Positive newborn screen of normal infant; mother is deficient	844
Glycosuria	839	Psychiatric presentations	844
Hair abnormalities	839	Psychotic behavior	844
HDL (lipoprotein) low	839	Ptosia	844
Hemolytic anemia	839	Pyloric stenosis, vomiting and erroneous diagnosis of	844
Hemophagocytosis (erythrophagocytosis)	839	Ragged red fibers	844
Hepatic carcinoma	839	Red urine	844
		Renal calculi	844

Renal cysts	844	Scoliosis	845
Renal Fanconi syndrome	845	Self-injurious behavior	845
Renal tubular acidosis (RTA)	845	Sensorineural deafness	845
Retinitis pigmentosa	845	Spastic paraparesis	845
Reye syndrome presentation	845	Stroke-like episodes	846
Reynaud syndrome	845	Subdural effusions	846
Rhabdomyolysis	845	Teeth colored	846
		Visual hallucinations	846
		Xanthomas	846

ACIDOSIS, HYPERCHLOREMIC

Diarrhea
 Acrodermatitis, enteropathica
 Infectious
 Lactase deficiency
 Sucrase deficiency
 Renal tubular acidosis (RTA)
 Cystinosis
 Fanconi syndrome
 Galactosemia
 Glucose, galactose malabsorption
 Hepatorenal tyrosinemia
 Mitochondrial electron transport defect
 Osteopetrosis and RTA
 Topamax

ALOPECIA

An (hypo) hidrotic ectodermal dysplasia
 Biotin deficiency
 Cartilage hair hypoplasia
 Conradi-Hünermann syndrome
 Multiple carboxylase deficiency – holocarboxylase synthetase and biotinidase deficiencies
 Trichorhexis nodosa-argininosuccinic aciduria
 Vitamin D-dependent rickets-receptor abnormalities

ANGIOKERATOMAS

Fabry disease
 Fucosidosis
 Galactosialidosis
 GM₁ gangliosidosis
 Sialidosis

APPARENT ACUTE ENCEPHALITIS

Glutaric aciduria type I
 NARP
 Propionic acidemia

ARTHRITIS

Alkaptonuria
 Farber disease
 Gaucher type I
 Gout-HPRT deficiency; PRPP overactivity
 Homocystinuria
 I cell disease
 Lesch-Nyhan disease
 Mucopolipidosis III
 Mucopolysaccharidosis I; II

BLACK PIGMENT DEPOSITION

Alkaptonuria – ochronosis
 Minocycline

BLEEDING TENDENCY

Abetalipoproteinemia
 α_1 -Antitrypsin deficiency
 Congenital disorders of glycosylation (CDG)
 Chediak-Higashi syndrome
 Fructose intolerance
 Gaucher disease
 Glycogenoses types I and IV
 Hermansky-Pudlak syndrome
 Peroxisomal disorder
 Tyrosinemia type I

CALCIFICATION OF BASAL GANGLIA

Albright syndrome
 Bilateral striato-pallido-dentate calcinosis
 Carbonic anhydrase II deficiency
 Cockayne syndrome
 Dihydrofolate reductase (DHFR) deficiency
 Down syndrome
 Familial progressive encephalopathy with calcification of the basal ganglia (Aicardi-Goutieres syndrome)
 GM₁ gangliosidosis
 Hallervorden-Spatz disease
 Krabbe leukodystrophy
 Lipoid proteinosis
 Microcephaly and intracranial calcification
 Mitochondrial cytopathies, MELAS, MERRF
 Neurofibromatosis
 Pterin defects
 Dihydropteridine reductase deficiency
 GTP cyclohydrolase I deficiency
 6-Pyruvoyletetrahydropterin synthase deficiency
 Sepiapterin reductase deficiency
 Spondyloepiphyseal dysplasia

CARDIOMYOPATHY

Congenital muscular dystrophy
 Disorders of fatty acid oxidation
 Electron transport chain abnormalities
 Glycogenosis type III
 Hemochromatosis
 D-2-Hydroxyglutaric aciduria
 3-Methylglutaconic aciduria
 Mucopolysaccharidosis
 Pompe disease

CARNITINE ESTERS

- C3 carnitine, elevated
 - Propionic acidemia
 - Methylmalonic acidemia
- C4-carnitine, elevated
 - Ethylmalonic encephalopathy (C-5 may also be increased)
 - Glutamate formiminotransferase (FTCD, MIM 229100) deficiency
 - IsobutyrylCoA dehydrogenase deficiency
 - SCAD (short chain acylCoA dehydrogenase) deficiency (C-5 may also be increased)
- C4OH, elevated
 - M/SCHAD (medium/short) chain acylCoA dehydrogenase deficiency
- C5-carnitine, elevated
 - Isovaleric acidemia
 - MethylbutyrylCoA (short branched chain) dehydrogenase deficiency
- C5OH carnitine, elevated
 - 3-Hydroxy-3-methylglutarylCoA lyase deficiency
 - 2-Oxothiolase deficiency
 - 3-MethylcrotonylCoA carboxylase deficiency (including maternal)
 - 2-Methyl-3-hydroxybutyrylCoA dehydrogenase deficiency
 - Mitochondrial acetoacetylCoA (3-oxo)thiolase deficiency
 - Multiple carboxylase deficiency (biotinidase, holocarboxylase synthetase)
 - Propionic acidemia (C3 always higher)
- C8, C6, C10, elevated
 - MCAD deficiency
- C14:1, elevated
 - VLCAD deficiency
- C16OH, C18:1OH, elevated
 - LCHAD deficiency

CATARACTS – LENTICULAR OPACITY

- Cerebrotendinous xanthomatosis
- Dystroglycanopathies
- Electron transport chain disorders
- Fabry disease
- Galactokinase deficiency
- Galactosemia
- Homocystinuria
- Hyperferritinemia-cataract syndrome
- Hyperornithinemia (ornithine aminotransferase deficiency)
- Lowe syndrome
- Lysinuric protein intolerance
- Mannosidosis
- Mevalonic aciduria

- Multiple sulfatase deficiency
- Neonatal carnitine palmitoyl transferase (CPT) II deficiency
- Δ^1 Pyrroline-5-carboxylate synthase deficiency
- Peroxisomal disorders
- Zellweger syndrome

CEREBRAL CALCIFICATION

- Abnormalities of folate metabolism
- Adrenoleukodystrophy
- Aicardi-Goutieres syndrome
- Biopterin abnormalities
- Biotinidase deficiency
- Carnitine palmitoyltransferase II deficiency
- Dihydrofolate reductase (DHFR) deficiency
- Cockayne syndrome
- GM₂ gangliosidosis
- Hypoparathyroidism
- Kearns-Sayre syndrome
- Krabbe disease
- MELAS
- Osteopetrosis and renal tubular acidosis (carbonic anhydrase II deficiency)
- L-2-Hydroxyglutaric aciduria
- Mitochondrial disorders

CEREBRAL VASCULAR DISEASE

- Fabry disease
- Familial hypocholesterolemia
- Homocystinuria
- Menkes disease
- Methylene tetrahydrofolate reductase deficiency
- Myocardial infarction

CEREBROSPINAL FLUID LYMPHOCYTOSIS

- Aicardi-Goutieres syndrome

CEREBROSPINAL FLUID PROTEIN ELEVATION

- Congenital disorders of glycosylation CDG
- L-2-Hydroxyglutaric aciduria
- Kearns-Sayre
- Krabbe disease
- MELAS (mitochondrial encephalomyopathy, lactic acidemia, and stroke-like episodes)
- MERFF (myoclonic epilepsy with ragged-red fibers)
- Metachromatic leukodystrophy
- Multiple sulfatase deficiency
- Neonatal adrenoleukodystrophy
- Refsum disease

CHERRY RED MACULAR SPOTS

Galactosialidosis
GM₁ gangliosidosis
Mucopolipidosis I
Multiple sulfatase deficiency
Niemann-Pick disease
Sandhoff disease
Sialidosis
Tay-Sachs disease

CHOLESTATIC JAUNDICE

Alagille syndrome
 α_1 -Antitrypsin deficiency
Bile acid synthesis defects
Byler disease (progressive familial intrahepatic cholestasis [PFIC1, BRIC1])
PFIC2 (bile salt excretory pump BSEP)
PFIC3 (MDR3)
Citrin deficiency
Cystic fibrosis
Dubin-Johnson syndrome
Fructose biphosphate aldolase (B) deficiency (HNF-1 β)
Hepatic nuclear factor 1 β gene mutations
Hepatorenal tyrosinemia
LCHAD deficiency
Mevalonic aciduria
Niemann-Pick disease
Niemann-Pick type C disease
Peroxisomal biogenesis disorders
Rotor syndrome
Tyrosinemia, hepatorenal

CHONDRODYSPLASIA PHENOTYPES

Conradi-Hünemann syndrome
Peroxisomal disorders
Warfarin embryopathy

CHRONIC PANCREATITIS

Hereditary (dominant) (with or without lysinuria (cystinuria)): with or without pancreatic lithiasis or portal vein thrombosis
With hyperparathyroidism in multiple endocrine adenomatosis syndrome
MELAS
Organic acidemia
Pearson syndrome
Regional enteritis (Crohn)
Trauma – pseudocyst

CIRRHOSIS OF THE LIVER

α_1 -Antitrypsin deficiency
Cholesteryl ester storage disease
Citrin deficiency (citrullinemia)
Cystic fibrosis
Congenital disorders of glycosylation
Defects of bile acid synthesis
Electron transport chain disorders
Fructose intolerance
Galactosemia
Gaucher disease
Glycogenosis types I and IV
Hemochromatosis
Hepatorenal tyrosinemia
Hypermethioninemia
Mitochondrial DNA depletion
Niemann-Pick type C
Peroxisomal disorders
Phosphoenolpyruvate carboxykinase deficiency
Progressive intrahepatic cholestasis
Pyruvate kinase deficiency
Thalassemia
Transaldolase deficiency
Wilson disease
Wolman disease

CORNEAL OPACITY

Cystinosis
Fabry
Fish eye disease (LCAT deficiency)
Galactosialidosis
GM₁ gangliosidosis
Hurler disease (MPS I)
I-cell disease
Mannosidosis
Mucopolipidosis III
Multiple sulfatase deficiency

CORPUS CALLOSUM AGENESIS

Adrenocorticotrophic hormone (ACTH) deficiency
Aicardi syndrome
Mitochondrial disorders (especially pyruvate dehydrogenase deficiency)
Nonketotic hyperglycinemia
Peroxisomal disorders

CREATINE KINASE – ELEVATED

Aldolase A deficiency
Carnitine palmitoyl transferase II deficiency
Disorders of fatty acid oxidation

Drugs – toxins, alcohol, statins
 Glutaric acidemia (I)
 Glycogenosis – III
 Glycogenosis – V – McArdle
 Glycogenosis – phosphofructokinase
 D-2-Hydroxyglutaric aciduria
 Inflammatory myopathy – dermatomyositis, polymyositis
 Infectious myositis
 Mevalonic aciduria
 Myoadenylate deaminase
 Muscular dystrophy – Duchenne, Becker
 3-Oxothiolase deficiency
 Oxphos abnormalities, MELAS, mtDNA depletion
 Traumatic muscle injury

DERMATOSIS

Acrodermatitic enteropathica
 Biotinidase deficiency
 Holocarboxylase synthetase deficiency

DIABETES MELLITUS – ERRONEOUS DIAGNOSIS

Congenital disorders of glycosylation
 Isovaleric acidemia
 Methylmalonic acidemia
 3-Oxothiolase deficiency
 Propionic acidemia

DIARRHEA

Abetalipoproteinemia
 Congenital chloride diarrhea
 Electron transport disorders
 Enterokinase deficiency
 Glucose galactose malabsorption
 Johansson-Blizzard syndrome
 Lactase deficiency
 Lysinuric protein intolerance
 Pearson syndrome
 Schwachman syndrome
 Sucrase deficiency
 Wolman disease

DISORDERS OF FOLATE METABOLISM OR TRANSPORT

Cerebral folate transport deficiency
 Dihydrofolate reductase deficiency (DHFR)
 Glutamate formiminotransferase deficiency
 Hereditary folate malabsorption
 Methionine synthase reductase deficiency (CbIE)

Methionine synthetase deficiency (CbIG)
 Methylene tetrahydrofolate reductase (MTHFR) deficiency

DRUGS OR OTHER TRIGGERS OF SYMPTOMS IN METABOLIC DISEASES

Infection
 Neuroleptics (Wilson disease, HPRT deficiency)
 Porphyrigenic drugs (imipramine, meprobamate), alcohol
 Surgery
 Valproate

DYSOSTOSIS MULTIPLEX

Galactosialidosis
 Generalized GM₁ gangliosidosis
 Hurler, Hurler-Scheie disease
 Hunter disease
 Maroteaux-Lamy disease
 Mucopolipidosis II, I-cell disease
 Mucopolipidosis III
 Multiple sulfatase deficiency
 Sanfilippo disease
 Sly disease

ECTOPIA LENTIS (DISLOCATION OF THE LENS)

Homocystinuria
 Hyperlysinemia
 Marfan syndrome
 Molybdenum cofactor deficiency
 Sulfite oxidase deficiency
 Weiss-Marchesani syndrome

EEG BURST SUPPRESSION PATTERN

Anesthesia – deep stages
 Anoxia, cerebral hypoperfusion
 Drug overdose (e.g. phenobarbital)
 Molybdenum cofactor deficiency
 Nonketotic hyperglycinemia
 Organic acidemia (neonatal encephalopathy-propionic acidemia)
 Sulfite oxidase deficiency

EXERCISE INTOLERANCE

Defects of glycogenolysis
 Disorders of fatty acid oxidation
 3-Oxothiolase deficiency

Mitochondrial disorders
Myoadenylate deaminase deficiency

FAILURE TO THRIVE

α_1 -Antitrypsin deficiency
Cobalamin C disease
Congenital disorders of glycosylation
Glycogenosis II
Hepatorenal tyrosinemia
Lysinuric protein intolerance
Methylmalonic acidemia
Mevalonic aciduria
Niemann-Pick disease
Pearson syndrome
Propionic acidemia
Wolman disease

FEVER SYNDROMES

Familial mediterranean fever
Hyperimmunoglobulin D syndrome (mevalonic aciduria)
Muckle-Wells syndrome (neonatal onset multisystem inflammatory disease syndrome)
Tumor necrosis factor receptor-associated periodic syndrome

GLYCOSURIA

Cystinosis
Diabetes mellitus
Hepatorenal tyrosinemia
Fanconi-Bickel syndrome – GLUT-2 mutations
Glycogen synthase deficiency
Pearson syndrome
Renal Fanconi syndrome
Wilson disease

HAIR ABNORMALITIES

Argininosuccinic aciduria
Björnstad syndrome (complex 3)
Kinky hair, photosensitivity, and impaired mental development
Menkes disease (pili torti, trichorrhexis nodosa, monilethrix)
Pili torti: isolated, MIM 261900
Pili torti with deafness or with dental enamel hypoplasia MIM 262000 (Björnstad syndrome)
Trichothiodystrophy: trichorrhexis nodosa, ichthyosis, and neurological abnormalities (Pollit syndrome) MIM 27550

HDL (LIPOPROTEIN) LOW

Lecithin cholesterol acyltransferase (LCAT) deficiency (fish eye disease)
Tangier disease
Hypoalphalipoproteinemia

HEMOLYTIC ANEMIA

Defects of glycolysis
5-Oxoprolinuria
Purine and pyrimidine disorders
Wilson disease

HEMOPHAGOCYTOSIS (ERYTHROPHAGOCYTOSIS)

Carnitine palmitoyl transferase I
Familial hemophagocytic lymphocytic histiocytosis (perforin deficiency, PRF1)
Gaucher disease
Hemochromatosis
Lysinuric protein intolerance
Niemann-Pick disease
Propionic acidemia
Wolman disease

HEPATIC CARCINOMA

α_1 -Antitrypsin deficiency
Galactosemia
Gaucher disease
Glycogen storage disease types I and IV
Hemochromatosis
Hepatorenal tyrosinemia
Progressive intrahepatic cholestasis
Thalassemia
Wilson disease

HEPATIC FAILURE – ACUTE

α_1 -Antitrypsin deficiency
Citrullinemia
Fatty acid oxidation disorders
Galactosemia
Hepatorenal tyrosinemia
Hereditary fructose intolerance
Neonatal hemochromatosis
Niemann-Pick types B and C
Wilson disease

HYDROPS FETALIS

Carnitine transporter deficiency
 Congenital disorders of glycosylation
 Farber disease (disseminated lipogranulomatosis)
 Galactosialidosis
 Glycogenosis type IV
 GM₁ gangliosidosis
 Gaucher disease
 Infantile free sialic acid storage disease (ISSD)
 Mucopolipidosis II – I-cell disease
 Neonatal hemochromatosis
 Niemann-Pick disease
 Niemann-Pick disease type C
 Pearson syndrome (anemia)
 Sialidosis
 Sly disease- β -glucuronidase deficiency
 Wolman disease

3-HYDROXYGLUTARIC ACIDURIA

Glutaryl CoA dehydrogenase deficiency
 Short chain hydroxyacyl CoA dehydrogenase deficiency
 Carnitine palmitoyltransferase I deficiency

HYPERAMMONEMIA

N-Acetylglutamate synthetase deficiency
 α_1 -Antitrypsin deficiency
 Argininemia
 Argininosuccinic aciduria
 Carbamoyl phosphate synthetase deficiency
 Carnitine palmitoyl transferase-II deficiency
 Chemotherapy-induced hyperammonemia
 Citrullinemia
 Fatty acid oxidation disorders
 HHH syndrome
 HMG CoA lyase deficiency
 Hyperthermia, malignant
 Isovaleric acidemia
 Lysinuric protein intolerance
 MCAD deficiency
 Methylmalonic acidemia
 Multiple carboxylase deficiency
 Ornithine transcarbamylase deficiency
 Pyruvate carboxylase deficiency
 Pyruvate dehydrogenase complex deficiency
 Propionic acidemia
 Transient hyperammonemia of the newborn
 Urinary tract infection – urea-splitting bacteria
 Valproate
 Wilson disease

HYPERCALCEMIA

Acute renal failure
 Endocrinopathies
 Thyrotoxicosis
 Adrenal insufficiency
 Pheochromocytoma
 Vasoactive intestinal peptide-producing tumor (VIPoma)
 Immobilization-associated
 Malignancy-associated hypercalcemia
 Milk-alkali syndrome
 Primary hyperparathyroidism and variants
 Sporadic primary hyperparathyroidism
 Familial primary hyperparathyroidism
 Associated with multiple endocrine neoplasia type 1 (MEN-1)
 Associated with multiple endocrine neoplasia type 2 (MEN-2)
 Familial benign hypocalciuric hypercalcemia
 Tertiary hyperparathyroidism
 In chronic renal failure
 After renal transplantation
 Associated with lithium therapy
 Sarcoidosis and other granulomatous diseases
 Vitamin A intoxication
 Vitamin D intoxication

HYPERTYROSINEMIA

Deficiency of 4-hydroxyphenylpyruvate dioxygenase
 Drug – toxin
 Hepatic infection
 Hepatorenal tyrosinemia
 Hyperthyroidism
 Oculocutaneous tyrosinemia
 Postprandial
 Scurvy
 Transient tyrosinemia of the newborn
 Treatment with NTBC
 Tyrosinemia type III

HYPOKETOTIC HYPOGLYCEMIA

Carnitine transporter deficiency
 CPT I deficiency
 HMG CoA lyase deficiency
 LCAD
 LCHAD
 MCAD
 VLCAD

HYPOPHOSPHATEMIA

Fanconi syndrome
Hyperparathyroidism
MELAS
Pearson
X-linked hypophosphatemic rickets

HYPOURICEMIA

Fanconi syndrome, cystinosis, any proximal renal tubular dysfunction
Isolated renal tubular defect (Dalmatian dog model)
Molybdenum cofactor deficiency
Phosphoribosyl pyrophosphate synthetase deficiency
Purine nucleoside phosphorylase deficiency
Wilson disease
Xanthine oxidase deficiency

ICHTHYOSIS

CHILD syndrome (congenital hemidysplasia, ichthyosis, and limb defects)
CDG (congenital disorders of glycosylation) type 1f
Gaucher disease
Krabbe disease
Multiple sulfatase deficiency
Refsum disease
Sjogren-Larsson syndrome
X-linked ichthyosis – steroid sulfatase deficiency

ICHTHYOSIS AND RETINAL DISEASE

Refsum syndrome
Sjogren-Larsson syndrome

INVERTED NIPPLES

Biopterin synthesis disorders
Citrullinemia
Congenital disorders of glycosylation
Glycogenosis 1b
Hyperphenylalaninemia
Isolated – dominant (MIM 163610)
Isovaleric acidemia
Menkes disease
Methylmalonic acidemia
Molybdenum cofactor deficiency
Niemann-Pick type C
Propionic acidemia
Pyruvate carboxylase deficiency
SCAD deficiency

VLCAD deficiency
Weaver syndrome

ISOLATED DEFICIENCY OF SPEECH AS PRESENTATION IN METABOLIC DISEASE

D-Glyceric aciduria
Electron transport chain disorder
Ethylmalonic aciduria
Histidinemia
3-Methylglutaconyl CoA hydratase

LACTIC ACIDEMIA

Electron transport chain disorders
Lues, congenital
MELAS
MERRF
Organic acidemia, e.g. propionic acidemia
Pyruvate carboxylase deficiency
Pyruvate dehydrogenase deficiency

LEIGH SYNDROME

Biotinidase deficiency
Electron transport chain abnormalities
Fumarase deficiency
3-Methylglutaconic aciduria
Pyruvate carboxylase deficiency
Pyruvate dehydrogenase complex deficiency
Sulfite oxidase deficiency

LEUKOPENIA WITH OR WITHOUT THROMBOPENIA AND ANEMIA

Abnormalities of folate metabolism
Isovaleric acidemia
Johansson-Blizzard syndrome
Methylmalonic acidemias
3-Oxothiolase deficiency
Pearson syndrome
Propionic acidemia
Schwachman syndrome
Transcobalamin II deficiency

MACROCEPHALY

Bannayan-Ruvalcaba-Riley syndrome
Canavan disease
Glutaric aciduria type I
Hurler disease
4-Hydroxybutyric aciduria

3-Hydroxy-3-methylglutaric aciduria
 L-2-Hydroxyglutaric aciduria
 Krabbe disease
 Mannosidosis
 Multiple acyl CoA dehydrogenase deficiency
 Multiple sulfatase deficiency
 Neonatal adrenoleukodystrophy
 Pyruvate carboxylase deficiency
 Tay-Sachs disease

MEGALOBlastic ANEMIA

B₁₂ deficiency – vegan or breastfed infant of vegan mother
 Cobalamin metabolic errors-methylmalonic acidemia and homocystinuria-Cbl C and D
 Folate metabolism, abnormalities of CblF cobalamin lysosomal transporter deficiency
 Dietary folate deficiency
 Folate malabsorption – hereditary – protein coupled folate transport (PCFT) deficiency
 Intestinal B₁₂ transport deficiency – Imerslund-Grasbeck-Cubilin deficiency
 Methylmalonic acidemia-homocystinuria-Cbl C+D
 Mevalonic aciduria
 Orotic aciduria
 Pearson syndrome
 Pernicious anemia – intrinsic factor deficiency
 Thiamine responsive megaloblastic anemia (OMIM 249270) (thiamine transporter [TRMA] mutation)
 Transcobalamin II deficiency

METABOLIC ACIDOSIS AND KETOSIS

Fabry disease
 Familial hypcholesterolemia
 Homocystinuria
 Isovaleric acidemia
 Menkes disease
 Methylcrotonyl CoA carboxylase deficiency
 Methylmalonic acidemia
 Multiple carboxylase deficiency
 Propionic acidemia

METHYLMALONIC ACIDURIA

B₁₂ deficiency, pernicious anemia, including autoimmune
 Cobalamin A
 Cobalamin C, D
 Imerslund-Gräsbeck – cobalamin enterocyte malabsorption
 Mut^o, Mut⁻
 Succinyl CoA synthase deficiency
 Transcobalamin II deficiency

MONGOLIAN SPOT – EXTENSIVE

GM₁ gangliosidosis
 Hurler syndrome
 Hunter syndrome
 Mannosidosis
 Niemann-Pick disease

MYOCARDIAL INFARCTION–CEREBRAL VASCULAR DISEASE

Fabry disease
 Familial hypercholesterolemia
 Homocystinuria
 Menkes disease
 Oxothiolase deficiency
 Propionic acidemia
 SCHAD deficiency

NEONATAL HEPATIC PRESENTATIONS IN METABOLIC DISEASES

α₁-Antitrypsin deficiency
 Cystic fibrosis
 Fructose intolerance
 Galactosemia
 Hemochromatosis
 Hepatorenal tyrosinemia
 Long-chain hydroxy-acyl CoA dehydrogenase deficiency
 Mitochondrial DNA depletion syndromes
 Niemann-Pick type C disease
 Wilson disease
 Wolman disease
 Sly disease

NEUTROPENIA

Isovaleric acidemia
 Methylmalonic aciduria
 Propionic acidemia
 Cartilage hair hypoplasia
 Cyclic neutropenia
 Leukemia
 Oxothiolase deficiency
 Splenic neutropenia
 Drug-induced agranulocytosis
 Idiopathic congenital neutropenias

ODD OR UNUSUAL ODOR

Dimethylglycinuria
 Glutaric aciduria type II

Hepatorenal tyrosinemia
Isovaleric acidemia
Maple syrup urine disease
Phenylketonuria
Trimethylaminuria
Treatment of urea cycle disorder with phenylacetate

OPTIC ATROPHY

ADP-ribosyl protein lyase deficiency
Adrenoleukodystrophy (ALD)
Biotinidase deficiency
Canavan disease
GM₁ gangliosidosis
Homocystinuria
Krabbe disease
Menkes disease
MERRF
Metachromatic leukodystrophy
3-Methylglutaconic aciduria, type III (Costeff)
Mevalonic aciduria
Mitochondrial energy metabolism, defects in – including
 Leber hereditary optic neuropathy (LHON)
Multiple sulfatase deficiency
NARP
Neonatal adrenoleukodystrophy
Sandhoff disease
Tay-Sachs disease

OROTIC ACIDURIA

UMP synthase deficiency (hereditary orotic aciduria)
Urea cycle defects – ornithine transcarbamylase deficiency,
 citrullinemia, argininosuccinic aciduria, arginemia
Purine nucleoside phosphorylase (PNP) deficiency
Phosphoribosylpyrophosphate (PRPP) synthetase
 deficiency

OSTEOPOROSIS AND FRACTURES

Adenosine deaminase deficiency
Gaucher disease
Glycogenesis I
Homocystinuria
I-cell disease
Infantile Refsum disease
Lysinuric protein intolerance
Menkes disease
Methylmalonic acidemia
Propionic acidemia

PAIN AND ELEVATED ERYTHROCYTE SEDIMENTATION RATE

Fabry disease
Familial hypercholesterolemia
Gaucher disease

PANCREATITIS

Carnitine palmitoyl transferase I deficiency
Carnitine palmitoyl transferase II deficiency
Cytochrome c oxidase deficiency
Glycogenesis type I
Glycogenesis 1 plus apoE2 type III hypertriglyceridemia
Hereditary dominant, with or without lysinuria; with or
 without pancreatic lithiasis or portal vein thrombosis
Homocystinuria
Hydroxymethylglutaryl CoA lyase deficiency
Hyperlipoproteinemia type IV
Isovaleric acidemia
Lesch-Nyhan disease
Lipoprotein lipase deficiency, also type IV
Lysinuric protein intolerance
Maple syrup urine disease
MELAS
Methylmalonic acidemia
Ornithine transcarbamylase deficiency
Pearson syndrome
Propionic acidemia
Regional enteritis (Crohn)
Trauma – pseudocyst
With hyperparathyroidism in multiple endocrine
 adenomatosis syndrome

PARALYSIS OF UPWARD GAZE

Leigh; Kearns-Sayre syndromes
Niemann-Pick type C
Peripheral neuropathy

PHOTOPHOBIA

Cystinosis
Oculocutaneous tyrosinemia

POLYCYSTIC KIDNEYS

Carnitine palmitoyl transferase II (CPT-II) deficiency
Congenital disorders of glycosylation
Multiple acyl CoA dehydrogenase deficiency, glutaric
 aciduria type II (GA II)
Zellweger syndrome

POSITIVE NEWBORN SCREEN OF NORMAL INFANT; MOTHER IS DEFICIENT

Carnitine transporter deficiency
 Holocarboxylase synthetase deficiency
 MethylcrotonylCoA carboxylase deficiency
 Very long chain acylCoA dehydrogenase deficiency
 Vitamin B₁₂ deficiency

PSYCHIATRIC PRESENTATIONS

Acute and recurrent confusion/psychosis
 Homocystine remethylation defects (MTHFR deficiency, Cblc)
 Porphyrias
 Urea cycle, especially OTC deficiency
 Chronic psychotic disease
 Adrenoleukodystrophy
 Homocystinurias
 Lysosomal diseases, metachromatic leukodystrophy, GM₂ gangliosidosis
 Wilson disease
 Late onset behavioral personality changes
 Cerebral tendinous xanthomatosis
 Ceroid lipofuscinosis
 Creatine transporter effect
 Homocystinurias
 4-Hydroxybutyric aciduria
 Mannosidosis, α and β
 Monoamine oxidase deficiency
 Niemann-Pick type C
 Nonketotic hyperglycinemia (late onset)
 Sanfilippo disease

PSYCHOTIC BEHAVIOR

Carbamoyl phosphate synthetase deficiency
 Cbl disease
 Ceroid lipofuscinosis
 Citrullinemia
 Hartnup disease
 Homocystinuria
 Hurler-Schie, Schie disease
 Krabbe disease
 Lysinuric protein
 Maple syrup urine disease
 MeFH₄ reductase deficiency
 Metachromatic leukodystrophy
 Mitochondrial disease (MELAS)
 Niemann-Pick type C disease
 Ornithine transcarbamylase deficiency
 Porphyria
 Sanfilippo disease
 Tay-Sachs, Sandhoff-late onset
 Wilson disease

PTOSIS

Kearn-Sayre syndrome
 MNGIE (mitochondrial neurogastrointestinal encephalomyelopathy)

PYLORIC STENOSIS, VOMITING AND ERRONEOUS DIAGNOSIS OF

Ethylmalonic-adipic aciduria
 Galactosemia
 HMG CoA lyase deficiency
 4-Hydroxybutyric aciduria
 D-2-Hydroxyglutaric aciduria
 Isovaleric acidemia
 Methylmalonic acidemia
 Molybdenum cofactor deficiency
 3-Oxothiolase deficiency
 Phenylketonuria
 Propionic acidemia

RAGGED RED FIBERS

Menkes disease
 Mitochondrial DNA mutations

RED URINE

Beets
 Congenital erythropoietic porphyria
 Drugs: ibuprofen, nitrofurantoin, pyridium, rifampicin
 Hematuria
 Hemoglobinuria
 Myoglobinuria
 Phenolphthalein
 Red diaper syndrome (*Serratia marcescens*)
 Red dyes (Monday morning disorder, rhodamine B)

RENAL CALCULI

APRT (adenosine phosphoribosyltransferase) deficiency
 Cystinuria
 HPRT deficiency–Lesch-Nyhan disease
 Oxaluria
 PRPP synthetase abnormalities
 Wilson disease
 Xanthine oxidase deficiency

RENAL CYSTS

Carnitine palmitoyl transferase deficiency
 Congenital disorders of glycosylation

RENAL FANCONI SYNDROME

Cystinosis
Electron transport defects
Galactosemia
Glycogenosis I and III
Hepatorenal tyrosinemia
Idiopathic
Lowe syndrome
Lysinuric protein intolerance
Wilson disease

RENAL TUBULAR ACIDOSIS (RTA)

Cystinosis
Fanconi syndrome
Galactosemia
Hepatorenal tyrosinemia
Mitochondrial electron transport defect
Osteopetrosis and RTA
Topamax

RETINITIS PIGMENTOSA

Abetalipoproteinemia
Congenital disorders of glycosylation
Ceroid lipofuscinosis
Hunter disease
Kearns-Sayre syndrome
LCHAD deficiency
Mevalonic aciduria
NARP
Peroxisomal biosynthesis disorders
Primary retinitis pigmentosa
Refsum disease
Sjogren-Larsson syndrome (fatty alcohol oxidoreductase deficiency)

REYE SYNDROME PRESENTATION

Gluconeogenesis, abnormalities of
Fatty acid oxidation, disorders of
Urea cycle, disorders of
Electron transport chain abnormalities
Fructose intolerance
Organic acidemias

REYNAUD SYNDROME

Fabry disease

RHABDOMYOLYSIS

Aldolase A (fructose biphosphate) deficiency
Disorders of fatty acid oxidation – LCHAD, VLCAD, CPTII
Drugs – methylenedioxymethylamphetamine (MDMA)
Glutaric acidemia
Glycogenosis
 V McArdle – myophosphorylase
 VII Tarui – phosphofructokinase
Glycolysis
 phosphoglycerate kinase
 phosphoglyceromutase
Infection – myositis
Ischemic injury
LPIN1 mutations
Mitochondrial DNA deletions (multiple)
Mitochondrial point mutations (MELAS)
Oxphos defects – complex I, complex II
Quail ingestion – coturnism
Toxin-tetanus, snake venom, alcohol, cocaine, bee venom

SCOLIOSIS

CDG
Homocystinuria

SELF-INJURIOUS BEHAVIOR

De Lange syndrome
Familial dysautonomia
Hepatorenal tyrosinemia
Lesch-Nyhan disease
Smith-Magenis syndrome
Sensory neuropathy

SENSORINEURAL DEAFNESS

Biotinidase deficiency
Canavan disease
Kearns-Sayre syndrome and other electron transport chain disorders
Peroxisomal disorders
PRPP synthetase abnormality
Refsum disease

SPASTIC PARAPARESIS

Argininemia
Biotinidase deficiency
HHH syndrome

Metachromatic leukodystrophy
 Pyroglutamic aciduria
 Sjögren-Larsson syndrome

STROKE-LIKE EPISODES

Carbamyl phosphate synthetase deficiency
 Chediak-Higashi syndrome
 Citrullinemia
 Congenital disorders of glycosylation
 Cystinosis
 Ethylmalonic aciduria
 Fabry disease
 Familial hypercholesterolemia
 Glutaric aciduria type I
 Homocystinuria
 Hydroxymethylglutaryl-CoA lyase deficiency
 Isovaleric acidemia
 MELAS and other mitochondrial disorders
 Menkes disease
 Methylcrotonyl CoA carboxylase deficiency
 Methylmalonic acidemia
 3-Methylene FH₄ reductase deficiency
 Multiple acyl CoA dehydrogenase deficiency
 Ornithine transcarbamylase deficiency and other urea cycle disorders
 Propionic acidemia
 Phosphoglycerate kinase deficiency
 Phosphorylase kinase deficiency
 Progeria
 Pyruvate carboxylase deficiency
 Pyruvate dehydrogenase deficiency
 Respiratory chain disorder
 Purine nucleoside phosphorylase deficiency
 Sulfite oxidase deficiency

SUBDURAL EFFUSIONS

Glutaric aciduria (I)
 D-2-Hydroxyglutaric aciduria
 Menkes disease
 Pyruvate carboxylase deficiency

TEETH COLORED

Amelogenesis imperfecta (dense white, lyonized)
 Dentinogenesis imperfecta (blue gray or brown)
 Fluorosis
 Hyperbilirubinemia (green)
 Hypothyroidism, congenital
 Porphyria, congenital erythropoietic (red, fluorescent)
 Tetracycline (brown, fluorescent)
 Tyrosinemia, oculocutaneous

VISUAL HALLUCINATIONS

Cobalamin C disease
 Maltase (acid) deficiency
 α-Mannosidosis
 Methylene tetrahydrofolate reductase (MTHFR) deficiency
 Niemann-Pick disease type C
 Propionic acidemia
 Tay-Sachs disease (late onset)
 Urea cycle defects

XANTHOMAS

Cerebrotendinous xanthomatosis
 Familial hypercholesterolemia
 Lipoprotein lipase deficiency
 Niemann-Pick disease
 Sitosterolemia

Index

Pages appearing in *italics* refer to tables and numbers appearing in **bold** refer to figures.

- AADC *see* aromatic L-amino acid decarboxylase (AADC)
- AB variant, GM₂ gangliosidosis *see* GM₂ activator deficiency
- ABCD1* gene 460, 464
- abdominal distension
- Gaucher disease 699, **699**, **700**
 - hepatorenal tyrosinemia 172, **172**
 - Niemann–Pick disease **710**, 711
 - von Gierke disease **430**
 - Wolman disease 733, **734**
- abdominal pain, lipoprotein lipase deficiency 649–50, 654
- acanthocytosis, Wolman disease 735
- acanthosis, oculocutaneous tyrosinemia 166
- ACAT1* gene 96, 99
- acetoacetate
- accumulation 9
 - elevated levels, acetoacetyl CoA thiolase deficiency 100
 - excretion 6
- acetoacetyl-CoA thiolase deficiency
- cytosolic 100
 - mitochondrial *see* mitochondrial acetoacetyl-CoA thiolase deficiency
- N-acetylaspargate (NAA)
- Canavan disease 811, 813, **815**, **815**
 - metachromatic leukodystrophy 763
- N-acetylaspargic aciduria 811
- N-acetylgalactosamine-4-sulfatase 597, **597**, 601
- N-acetylgalactosamine-4-sulfatase deficiency *see* Maroteaux–Lamy disease
- N-acetylgalactosamine-6-sulfatase deficiency 588, **588**, 593–4
- see also* Morquio syndrome
- N-acetylglucosamine-6-sulfatase deficiency 580, **581**, 585
- see also* Sanfilippo disease
- α-N-acetylglucosaminidase deficiency 580, **581**, 585
- see also* Sanfilippo disease
- N-acetylglucosaminyl-1-phosphotransferase 613, **614**, 617, 621, 625
- N-acetylglucosaminyl-1-phosphotransferase deficiency 613–14, **614**, 617, 621
- see also* I-cell disease; mucopolipidosis III
- N-acetylglutamate synthase (NAGS) 226, **226**
- N-acetyltransferase deficiency 580, **581**, 585
- see also* Sanfilippo disease
- acid β-glucosidase 698, **699**, 703
- deficiency *see* Gaucher disease
- acid lipase 733, **733**, 737
- deficiency 733, **733**, 736, 737
- acidemia
- isovaleric *see* isovaleric acidemia
 - lactic *see* lactic acidemia
 - methylmalonic *see* methylmalonic acidemia
 - mevalonic 5
 - propionic *see* propionic acidemia
 - see also* organic acidemias
- acidosis
- biotinidase deficiency 50
 - holocarboxylase synthetase deficiency 41, 42
 - hyperammonemia 192, **192**
 - hyperchloremic 835
 - lactic *see* lactic acidosis
 - maple syrup urine disease 154, 155, 156
 - 3-methylcrotonyl CoA carboxylase deficiency 75
 - mitochondrial acetoacetyl-CoA thiolase deficiency 96, 98
 - renal tubular *see* renal tubular acidosis (RTA)
 - see also* metabolic acidosis
- aciduria
- N-acetylaspargic 811
 - argininosuccinic *see* argininosuccinic aciduria
 - dicarboxylic *see* dicarboxylic aciduria
 - ethylmalonic 303, 820, 823
 - glutaric, type I *see* glutaric aciduria, type I
 - glutaric, type II *see* multiple acyl CoA dehydrogenase deficiency (MADD)
 - 3-hydroxy-3-methylglutaric (HMG) 325, 338
 - 4-hydroxybutyric *see* 4-hydroxybutyric aciduria
 - D-2-hydroxyglutaric *see* D-2-hydroxyglutaric aciduria
 - L-2-hydroxyglutaric *see* L-2-hydroxyglutaric aciduria
 - 4-hydroxyisobutyric 83
 - lactic *see* lactic aciduria
 - methylmalonic *see* methylmalonic aciduria
 - mevalonic *see* mevalonic aciduria
 - orotic *see* orotic aciduria
 - pipecolic, neonatal
 - adrenoleukodystrophy 469, 476
 - pyroglutamic 338
- acrocyanosis, ethylmalonic encephalopathy 820–1, **821**
- acrodermatitis enteropathica
- biotinidase deficiency **49**, 50
 - cobalamin C disease 35
 - holocarboxylase synthetase deficiency 41
 - lysine protein intolerance 236, **236**
 - ornithine transcarbamylase deficiency 201
- acroparesthesias, Fabry disease 660
- acute demyelinating encephalomyelitis (ADEM) 390
- acute fatty liver of pregnancy (AFLP)
- carnitine palmitoyl transferase I deficiency 269
 - long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 296, 298
- acute neuronopathic Gaucher disease (type II Gaucher disease) 698, 698, 702
- acyl CoA dehydrogenases **281**, **281**
- acylcarnitine profiles
- carnitine palmitoyl transferase I deficiency **270**
 - carnitine palmitoyl transferase II deficiency, lethal neonatal 273, **274**, 275
 - carnitine–acylcarnitine translocase deficiency 263, **263**
 - fatty acid oxidation disorders 247, **250**, 250–1
 - 3-hydroxy-3-methylglutaryl CoA lyase deficiency 330, **330**
 - 3-hydroxyacyl CoA dehydrogenase deficiency 310
 - D-2-hydroxyglutaric aciduria 82
 - lactic acidemias 338
 - long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 297, **298**
 - medium-chain acyl CoA dehydrogenase deficiency 282, 284, **284**, 285, 286
 - mitochondrial acetoacetyl-CoA thiolase deficiency 98, **98**
 - multiple acyl CoA dehydrogenase deficiency 320, **320**
 - organic acidemias 5
 - short/branched chain acyl CoA dehydrogenase deficiency 314
 - short-chain acyl CoA dehydrogenase deficiency 304, **305**
- adenine phosphoribosyl-transferase (APRT) 498, **498**, 499
- adenine phosphoribosyl-transferase (APRT) deficiency 498–500
- clinical abnormalities **498**, 499
 - genetics and pathogenesis **498**, 499–500
 - treatment 500
- adenosine deaminase (ADA) 507, **507**, 509
- adenosine deaminase (ADA) deficiency 507–11
- clinical abnormalities 507–9, **510**
 - genetics and pathogenesis 509–10
 - treatment 510–11

- adenosine levels, adenosine deaminase deficiency 509
- adenosine triphosphatase (ATP)-binding cassette transporter A1 (ABACA1), Krabbe disease 722
- adenosine triphosphatase (ATP)-binding cassette transporters, adrenoleukodystrophy 464
- adenosylcobalamin 19, 20, 26
- S-adenosylhomocysteine (SAH) hydrolase 510
- adenylosuccinate levels, adenosine deaminase deficiency 515
- adenylosuccinate lyase (ASL) 514, 514, 516
- adenylosuccinate lyase (ASL) deficiency 514–16
- clinical abnormalities 515, 515–16
- genetics and pathogenesis 514, 516
- treatment 516
- adipic acid 6, 6, 7
- mitochondrial acetoacetyl-CoA thiolase deficiency 98
- multiple acyl CoA dehydrogenase deficiency 320
- adrenal calcification, Wolman disease 733, 734–5, 735, 736
- adrenal insufficiency
- adrenoleukodystrophy 461
- neonatal adrenoleukodystrophy 473–4
- adrenoleukodystrophy (ALD) 459–65
- clinical abnormalities 460, 460–3, 461, 462, 463
- genetics and pathogenesis 459, 463–4
- neonatal *see* neonatal adrenoleukodystrophy
- treatment 464–5
- adrenomyeloneuropathy 459
- adult galactosialidosis 753, 755, 756
- adult GM₁ gangliosidosis 670–1, 673
- adult GM₂ gangliosidosis 679, 681
- AGAT deficiency *see* arginine:glycine amidinotransferase (AGAT) deficiency
- aggression
- adrenoleukodystrophy 460
- Hunter disease 575
- 4-hydroxybutyric aciduria 93
- Lesch–Nyhan disease 485, 486
- Sanfilippo disease 583
- alanine aminotransferase (ALT)
- carnitine palmitoyl transferase I deficiency 269
- carnitine transporter deficiency 254
- carnitine–acylcarnitine translocase deficiency 263
- HHH syndrome 231
- mitochondrial DNA depletion 404, 405, 406
- Niemann–Pick disease 709
- alanine levels
- argininosuccinic aciduria 219
- carbaryl phosphate synthetase deficiency 207
- citrullinemia 212
- HHH syndrome 231
- hyperammonemia 193, 195
- lysine protein intolerance 237
- ornithine transcarbamylase deficiency 199
- pyruvate carboxylase deficiency 348, 351
- alanine loading
- HHH syndrome 231, 231
- ornithine transcarbamylase deficiency 200
- Alder granules 601
- Alder–Reilly inclusions 607, 774
- ALDH5A1 gene 89
- alkaline phosphatase levels
- cystinosis 533
- Niemann–Pick disease 709
- alkaptonuria 105–9
- clinical abnormalities 105–8, 106, 107, 108
- genetics and pathogenesis 106, 108–9, 109
- treatment 105, 109
- allopurinol test, ornithine transcarbamylase deficiency 200
- allopurinol therapy
- adenine phosphoribosyl-transferase deficiency 500
- Lesch–Nyhan disease 494
- phosphoribosylpyrophosphate synthetase abnormalities 505
- von Gierke disease 434
- alopecia 41–2, 835
- an(hypo)hidrotic ectodermal dysplasia 42
- argininosuccinic aciduria 216, 217, 217, 218
- biotinidase deficiency 49, 50, 50, 54
- cartilage hair hypoplasia 42
- holocarboxylase synthetase deficiency 41, 42
- lysine protein intolerance 235, 236
- methylmalonic acidemia 21
- propionic acidemia 11
- vitamin D receptor abnormalities 42
- see also* trichorrhexis nodosa
- Alpers syndrome 404, 405, 405, 408–9
- alpha-fetoprotein, hepatorenal tyrosinemia 173, 175, 176
- ALT *see* alanine aminotransferase (ALT)
- Alzheimer type II cells
- methylmalonic acidemia 23
- mitochondrial DNA depletion 407
- amino acid metabolism disorders 105–85
- alkaptonuria *see* alkaptonuria
- biogenic amines *see* biogenic amine abnormalities
- hepatorenal tyrosinemia *see* hepatorenal tyrosinemia
- homocystinuria *see* homocystinuria
- hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin *see* hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin
- maple syrup urine disease *see* maple syrup urine disease (MSUD)
- nonketotic hyperglycinemia *see* nonketotic hyperglycinemia
- oculocutaneous tyrosinemia *see* oculocutaneous tyrosinemia
- phenylketonuria *see* phenylketonuria (PKU)
- aminoaciduria
- cystinosis 533
- galactosemia 418
- Hartnup disease 540, 540, 541, 541, 542
- hepatorenal tyrosinemia 173, 174, 175
- HHH syndrome 232
- lysine protein intolerance 237
- von Gierke disease 431
- γ-aminobutyric acid *see* GABA (γ-aminobutyric acid)
- δ-aminolevulinic acid, hepatorenal tyrosinemia 171, 175, 176
- 3-aminopiperid-2-one 232
- aminotransferases *see* transaminases
- amylase-1,6-glucosidase 447, 447, 452
- deficiency *see* glycogenosis type III
- amyloidosis, von Gierke disease 432
- Andersen disease 426, 426
- anemia
- cobalamin C disease 34
- dihydrofolate reductase deficiency 141
- galactosialidosis 754
- Gaucher disease 699, 701
- hemolytic 839
- lactic acidemias 344
- lipoprotein lipase deficiency 651
- lysine protein intolerance 236, 238
- methylmalonic acidemia 19, 19, 24
- mevalonic aciduria 645
- multiple acyl CoA dehydrogenase deficiency 317
- Niemann–Pick disease 710, 711
- orotic aciduria 518, 519
- Pearson syndrome 398, 399, 401, 402
- propionic acidemia 11
- Wolman disease 735
- see also* megaloblastic anemia
- angiokeratomas 835
- Fabry disease 659, 660, 660
- fucosidosis 741, 741
- galactosialidosis 754, 755, 755
- GM₁ gangliosidosis 668
- an(hypo)hidrotic ectodermal dysplasia 42, 50
- animal models
- carnitine transporter deficiency 256
- cystinuria 528
- familial hypercholesterolemia 635–6
- fucosidosis 742
- GM₁ gangliosidosis 674
- Hurler syndrome 563
- maple syrup urine disease 155
- Maroteaux–Lamy disease 602
- Niemann–Pick type C disease 721
- oculocutaneous tyrosinemia 167
- Sandhoff disease 691
- anion gap 3
- hyperammonemia 192
- propionic acidemia 9
- ankle clonus
- carbaryl phosphate synthetase deficiency 206
- ethylmalonic encephalopathy 820
- HHH syndrome 230, 231
- multiple sulfatase deficiency 770
- anorexia
- argininemia 223
- argininosuccinic aciduria 216
- citrullinemia 210
- cobalamin C disease 34
- fructose-1,6-diphosphatase deficiency 355
- galactosemia 415
- glutaric aciduria type I 66
- methylmalonic acidemia 20
- Niemann–Pick disease 710
- antithrombin III levels
- CDG type Ia 784
- CDG type Ib 788
- CDG type If 791
- CDG type Ig 793
- CDG type Iq 794

- α 1-antitrypsin (AT) 803, 806–7
 α 1-antitrypsin (AT) deficiency 803–8
 clinical abnormalities 803–6, **804**, **805**, **806**
 diagnosis 806
 genetics and pathogenesis 806, 806–7
 treatment 807–8
 aortic valve disease
 familial hypercholesterolemia 633
 Maroteaux–Lamy disease 599
 Morquio syndrome 591
 mucopolidosis III 625
 Scheie disease 567
 apnea
 carbamyl phosphate synthetase
 deficiency 206
 carnitine–acylcarnitine translocase
 deficiency 261
 CDG type If 792
 citrullinemia 211
 3-hydroxy-3-methylglutaryl CoA lyase
 deficiency 326
 D-2-hydroxyglutaric aciduria 80
 hyperammonemia 193
 NARP 390
 apolipoprotein B-100 (APOB), mutations 631,
 634–5, 636
 apolipoprotein C-II deficiency 653, 654
 apraxia, adrenoleukodystrophy 460
 arachnodactyly, homocystinuria 146, **147**
 arginase 223, **223**, 225
 arginine **525**
 arginine excretion, cystinuria 525, 527, 528
 arginine levels
 argininemia 225
 carbamyl phosphate synthetase
 deficiency 207
 citrullinemia 212
 multiple acyl CoA dehydrogenase
 deficiency 320
 arginine therapy
 argininosuccinic aciduria 220, **220**
 citrullinemia **213**, 213–14
 hyperammonemia 193, 194, 194–5
 MELAS 379
 ornithine transcarbamylase deficiency 201
 arginine:glycine amidinotransferase (AGAT)
 deficiency 827, **827**
 clinical abnormalities 828, 829, 829
 genetics and pathogenesis 828, 830
 treatment 830
 argininemia 193, 223–7
 clinical abnormalities 223–5, **224**
 genetics and pathogenesis 225–6, **226**
 treatment 226–7
 argininosuccinic aciduria 193, 194, 216–20
 clinical abnormalities 42, 216–19, **217**, **218**
 genetics and pathogenesis **216**, 219–20
 treatment 220, **220**
 arginosuccinate lyase (arginosuccinase) 216,
 216, 219, 220
 arginosuccinate synthetase 210, **210**, 212, 213
 defective activity *see* citrullinemia
 aromatic L-amino acid decarboxylase
 (AADC) **137**
 deficiency 136–9, **137**, **138**, 138
 arrhythmia *see* cardiac arrhythmia
 arthralgia, mevalonic aciduria 643, 645
 arthritis 835
 alkaptonuria 105, 107–8, **108**, 109
 familial hypercholesterolemia 633–4
 gouty *see* gouty arthritis
 arylsulfatase A (ASA) 760, **760**, 763, 769
 arylsulfatase A (ASA) deficiency 760–1, 763–4,
 770, 775, 776
 arylsulfatase A (ASA) pseudodeficiency 760–1,
 763, 764
 ascites
 α 1-antitrypsin (AT) deficiency 804
 CDG type If 792
 CDG type Ih 793
 galactosemia 415, **416**
 GM₁ gangliosidosis 667
 hepatorenal tyrosinemia 172, **173**
 Wolman disease 734, 735
 ascorbic acid, alkaptonuria management 109
 aspartate aminotransferase (AST)
 carnitine palmitoyl transferase I
 deficiency 269
 carnitine transporter deficiency 254
 carnitine–acylcarnitine translocase
 deficiency 263
 HHH syndrome 231
 mitochondrial DNA depletion 404, 405, 406
 Niemann–Pick disease 709
 aspartic acid levels
 carbamyl phosphate synthetase
 deficiency 207
 citrullinemia 212
 aspartic acid supplementation, pyruvate
 carboxylase deficiency 352
 aspartoacylase 811, **811**, 814–15
 aspartoacylase deficiency *see* Canavan disease
 astereognosis, adrenoleukodystrophy 460
 ataxia
 argininosuccinic aciduria 217, 218
 biotinidase deficiency 50
 CDG type Ic 789
 CDG type Ie 791
 CDG type IIh 795
 CDG type IIIi 795
 chronic/adult GM₁ gangliosidosis 670
 citrullinemia 212
 cobalamin C disease 34
 congenital disorders of glycosylation, type
 Ia 782, 783
 cytosolic acetoacetyl CoA thiolase
 deficiency 100
 Hartnup disease 541, 542
 HHH syndrome 229, 230
 4-hydroxybutyric aciduria 89, 91, 92
 L-2-hydroxyglutaric aciduria 86, 87
 isovaleric acidemia 58, 59
 juvenile GM₂ gangliosidosis 681
 Kearns–Sayre syndrome 394
 lactic acidemias 343
 late infantile/juvenile GM₁
 gangliosidosis 669
 MELAS 376, 377
 MERRF disease 383
 metachromatic leukodystrophy 761, 762
 mevalonic aciduria 643, 644
 mitochondrial acetoacetyl-CoA thiolase
 deficiency 97
 mitochondrial DNA depletion 406, 408
 multiple sulfatase deficiency 773
 NARP 388, 389, 390
 Niemann–Pick type C disease 719, 720
 ornithine transcarbamylase deficiency 199
 pyruvate dehydrogenase complex
 deficiency 361
 Sandhoff disease 688
 atherosclerosis, familial
 hypercholesterolemia 632
 ATP7A 546, 549
 attention deficit disorder
 medium-chain acyl CoA dehydrogenase
 deficiency 283
 MELAS 376
 Austin disease *see* multiple sulfatase deficiency
 (MSD)
 autistic behavior
 adenylosuccinate lyase deficiency 514, 515
 cerebral creatine deficiency 828, 830
 autoimmune disease, adenosine deaminase
 deficiency 508
 azidothymidine (AZT) toxicity, mitochondrial
 DNA depletion 409

 B₁₂ deficiency 6, 19, 19, 34
 B₁₂-responsive methylmalonic acidemia 27
 Babinski responses
 Canavan disease 813
 ethylmalonic encephalopathy 820
 fucosidosis 741
 GM₂ activator deficiency 694
 HHH syndrome 230, 231
 D-2-hydroxyglutaric aciduria 80
 L-2-hydroxyglutaric aciduria 86
 hyperphenylalaninemia and defective
 metabolism of tetrahydrobiopterin
 125
 Lesch–Nyhan disease 484
 phenylketonuria 115
 propionic acidemia 10
 baclofen, glutaric acidemia I management 72
 bacterial metabolites, intestinal 6, 6–7
 basal ganglia
 biotinidase deficiency 52
 calcification 835
 glutaric aciduria type I 67, 69, 72
 GM₁ gangliosidosis, chronic/adult 670
 GM₁ gangliosidosis, infantile 668
 D-2-hydroxyglutaric aciduria 81
 hyperphenylalaninemia and defective
 metabolism of tetrahydrobiopterin 127
 isovaleric acidemia 59, **60**
 Kearns–Sayre syndrome 394
 Lesch–Nyhan disease 484, 485, 494
 MELAS 375, **375**
 methylmalonic acidemia 22–3, **23**
 methylmalonic aciduria and
 homocystinuria 35–6
 NARP 390, 391
 propionic acidemia 10, 11
 pyruvate dehydrogenase complex
 deficiency 361
 behavior problems
 adrenoleukodystrophy 460
 Hunter disease 575
 Lesch–Nyhan disease 483, 484, 485–6, 493,
 494
 Niemann–Pick type C disease 720
 ornithine transcarbamylase deficiency 199
 phenylketonuria 115, 119

- behavior problems – *cont.*
 Sanfilippo disease 581, 583
see also psychotic behavior; self-injurious behavior
- benzoate therapy **194**
 argininemia 226–7
 hyperammonemia 194, 194–5
 nonketotic hyperglycinemia 185
 ornithine transcarbamylase deficiency 201, 201
- bezafibrate therapy, carnitine palmitoyl transferase II deficiency, late onset 279
- bicarbonate levels, glutaric aciduria type I 66
- bicarbonate therapy, lactic acidosis 344
 pyruvate dehydrogenase complex deficiency 364
- D-bifunctional protein deficiency 472–3
- bilirubinemia, α 1-antitrypsin (AT) deficiency 804
- biocytin **47, 48**
 urine 53
- biogenic amine abnormalities 136–42, **138**
 aromatic L-amino acid decarboxylase deficiency (AADC) 136–9, **137, 138, 138**
 dihydrofolate reductase deficiency 141–2
 tyrosine hydroxylase deficiency 139–40
- biopterin *see* tetrahydrobiopterin (BH₄)
- biotin **47, 48**
- biotin deficiency 42
- biotin therapy
 biotinidase deficiency 51, 54
 holocarboxylase synthetase deficiency 41, 44, **44**
 propionic acidemia 14
- biotinidase 47, 48, **48, 49**
- biotinidase assay, lactic acidemias 340
- biotinidase deficiency 40, 47–54
 clinical abnormalities 41, **48**, 48–52, **49, 51**
 genetics and pathogenesis 52–3
 treatment 54
- bisphosphonate therapy, Gaucher disease 705
- biting, Lesch–Nyhan disease 483, 485, **485, 486, 486**
- bizarre behavior
 ornithine transcarbamylase deficiency 199
see also behavior problems
- black pigment deposition 835
 alkaptonuria 107, **107**
- bleeding 835
 α 1-antitrypsin (AT) deficiency 804
 Gaucher disease 700, 701
 hepatorenal tyrosinemia 173
 Niemann–Pick disease 711
 von Gierke disease 431, 432, 433, 434
see also gastrointestinal bleeding; hemorrhages
- blepharoconjunctivitis, biotinidase deficiency 49
- blindness
 Canavan disease 813
 GM₂ activator deficiency 694
 Hurler–Scheie disease 568
 D-2-hydroxyglutaric aciduria 80
 Krabbe disease 728
 lactic acidemias 344
 methylmalonic acidemia 23
 multiple sulfatase deficiency 770
- Sandhoff disease 687
 Scheie disease 567
 Tay–Sachs disease 680
see also visual impairment
- Bombay blood phenotype 794
- bone marrow abnormalities
 cystinosis 535, **535**
 fucosidosis 742, **742**
 lysinuric protein intolerance 236–7
 Niemann–Pick disease 711, **713**
 Niemann–Pick type C disease 720
 Pearson syndrome 399, **400, 401**
 Sly disease 608
 Wolman disease 735, **736**
- bone marrow transplantation
 adenosine deaminase deficiency 510
 adrenoleukodystrophy 464
 fucosidosis 742
 Hurler disease 564
 Krabbe disease 730
 Maroteaux–Lamy disease 602
 metachromatic leukodystrophy 764
 mevalonic aciduria 646
 multiple sulfatase deficiency 776
 Niemann–Pick disease 711, 715
 Sandhoff disease 691
 Sanfilippo disease 585
 Sly disease 608
 Wolman disease 737
- bony dysplasia
 adenosine deaminase deficiency 508–9
see also skeletal abnormalities
- bradycardia, L-2-hydroxyglutaric aciduria 87
- bradykinesia, hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin 125
- brain tumors, L-2-hydroxyglutaric aciduria 87
- brainstem auditory evoked responses (BAERs), metachromatic leukodystrophy 763
- branched-chain oxoacid dehydrogenase complex 152, **152, 153, 156**
- branched-chain oxoacid dehydrogenase deficiency 371
- branched-chain oxoacid dehydrogenase
 deficiency 371
 urine disease (MSUD)
- bromocriptine therapy, aromatic L-amino acid decarboxylase deficiency 139
- butanone, mitochondrial acetoacetyl-CoA thiolase deficiency 98
- butyldeoxynojirimycin therapy, Niemann–Pick type C disease 722
- cachexia, Niemann–Pick disease 710, 711, **713**
- calcification
 adrenal, Wolman disease 733, 734–5, **735, 736**
 basal ganglia 835
 cerebral *see* cerebral calcification
- calculi
 renal *see* renal calculi
 urinary tract *see* urinary tract calculi
- Canavan disease 811–16
 clinical abnormalities 811–14, **812, 813, 814**
 genetics and pathogenesis **811, 814, 814–15, 815**
 reproductive options 815–16
 treatment 816
- cancer susceptibility, Gaucher disease 701
- candidiasis
 adenosine deaminase deficiency 508, 509
 biotinidase deficiency 50
- carbamazepine therapy, Fabry disease 662
- carbamyl phosphate synthetase (CPS) 205, **205, 207**
- carbamyl phosphate synthetase (CPS)
 deficiency 192, 193, 199, 205–7
 clinical abnormalities 205–7, **206**
 genetics and pathogenesis **205, 207**
 treatment 194, 207
- carbamylglutamate therapy
 carbamyl phosphate synthetase deficiency 207
 hyperammonemia 195
 methylmalonic acidemia 28
- carbidopa therapy
 hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin 131
 tyrosine hydroxylase deficiency 140
- carbohydrate content, common medications 357
- carbohydrate metabolism disorders 415–53
 galactosemia *see* galactosemia
 glycogen storage diseases *see* glycogen storage diseases
- carbohydrate-deficient glycoprotein syndromes *see* congenital disorders of glycosylation (CDG)
- carboxylase assay, lactic acidemias 340
- cardiac arrhythmia
 carnitine palmitoyl transferase II deficiency, late onset 278
 carnitine palmitoyl transferase II deficiency, lethal neonatal 273
 carnitine–acylcarnitine translocase deficiency 261, 262
 Fabry disease 661
 medium-chain acyl CoA dehydrogenase deficiency 283
 Pompe disease 444
- cardiac conduction
 Kearns–Sayre syndrome 394, **394**
see also electrocardiogram (ECG)
- cardiac disease
 alkaptonuria 108
 carnitine transporter deficiency 254, 255, 258
 carnitine–acylcarnitine translocase deficiency 261, 262
 Fabry disease 661
 familial hypercholesterolemia 632, 634
 Hunter disease 575
 Hurler disease 560
 Hurler–Scheie disease 568
 Maroteaux–Lamy disease 599–600
 phenylketonuria 115
 Scheie disease 567
see also specific cardiac diseases
- cardiac failure
 Hurler disease 560
 Maroteaux–Lamy disease 600
 Pompe disease 439, 441
- cardiomegaly
 carnitine palmitoyl transferase II deficiency, lethal neonatal 273
 Fabry disease 661
 multiple acyl CoA dehydrogenase

- deficiency 319
- Pompe disease 439, **440**, 441
- cardiomyopathy 835
- carnitine transporter deficiency 254, 255, 258
- carnitine–acylcarnitine translocase
 - deficiency 261, 264
- CDG type Ik 793
- CDG type Im 793
- CDG type Io 794
- congenital disorders of glycosylation, type Ia 783
- fatty acid oxidation disorders 247
- glycogenosis type III 450
- GM₁ gangliosidosis 668
- hepatorenal tyrosinemia 174
- Hurler disease 560
- D-2-hydroxyglutaric aciduria 80
- Kearns–Sayre syndrome 394
- lactic acidemias 344
- long-chain L-3-hydroxyacyl CoA
 - dehydrogenase deficiency 295, 296, 297, 298
- medium-chain acyl CoA dehydrogenase
 - deficiency 281, 283, 286
- MELAS 377, 378
- mitochondrial acetoacetyl-CoA thiolase
 - deficiency 96
- multiple acyl CoA dehydrogenase
 - deficiency 319
- Pompe disease 439, 444
- propionic acidemia 13
- short-chain 3-hydroxyacyl CoA
 - dehydrogenase deficiency 309
- Sly disease 607
- very long-chain acyl CoA dehydrogenase
 - deficiency 289, 290, 291, 292
- carnitine deficiency 253
- 3-methylcrotonyl CoA carboxylase
 - deficiency 75
- mitochondrial acetoacetyl-CoA thiolase
 - deficiency 96
- multiple acyl CoA dehydrogenase
 - deficiency 319, 320, 321
- carnitine esters 836
- carnitine levels 255
- carnitine palmitoyl transferase I
 - deficiency 269
- carnitine palmitoyl transferase II deficiency, lethal neonatal 276
- carnitine transporter deficiency 254, 255, 255, 256
- medium-chain acyl CoA dehydrogenase
 - deficiency 283–4, 286
- multiple acyl CoA dehydrogenase
 - deficiency 320
- carnitine metabolism 260
- carnitine palmitoyl transferase (CPT) I 257, 267, **267**, 268, 271
- carnitine palmitoyl transferase (CPT) I
 - deficiency 257, 267–71
 - clinical abnormalities **268**, 268–9, **270**
 - genetics and pathogenesis 269–71
 - treatment 271
- carnitine palmitoyl transferase (CPT) II 275
- carnitine palmitoyl transferase (CPT) II
 - deficiency 273, 277
- late onset 277–9
- lethal neonatal 273–6, **274**, **275**, 276, 277
- carnitine therapy
- carnitine transporter deficiency 254, 258
- carnitine–acylcarnitine translocase
 - deficiency 265
- cystinosis 537
- glutaric acidemia I 71, 71
- isovaleric acidemia 61
- long-chain L-3-hydroxyacyl CoA
 - dehydrogenase deficiency 299
- medium-chain acyl CoA dehydrogenase
 - deficiency 283, 286–7
- 3-methylcrotonyl CoA carboxylase
 - deficiency 77
- methylmalonic acidemia 28
- mitochondrial acetoacetyl-CoA thiolase
 - deficiency 100
- mitochondrial DNA depletion
 - management 409
- multiple acyl CoA dehydrogenase
 - deficiency 321
- propionic acidemia 14, 15, **15**
- short/branched chain acyl CoA
 - dehydrogenase deficiency 314
- very long-chain acyl CoA dehydrogenase
 - deficiency 292
- carnitine transporter **253**, 253–4, 256
- carnitine transporter deficiency (CTD) 251, 253–8
 - clinical abnormalities **254**, 254–5, **256**
 - genetics and pathogenesis 255, 255–8, **257**
 - treatment 258
- carnitine–acylcarnitine translocase 257, 260, **260**, 264
- carnitine–acylcarnitine translocase
 - deficiency 260–5
 - clinical abnormalities **261**, 261–4, **262**, **263**
 - genetics and pathogenesis **262**, 264–5
 - treatment 265
- carnitine-independent oxidation, peroxisomes **469**
- carpal tunnel syndrome
 - Maroteaux–Lamy disease 598–9
 - Scheie disease 567
- cartilage hair hypoplasia 42, 842
- cataracts 836
 - Fabry disease 661
 - galactosemia 417, 419, 421, 422
 - α-mannosidosis 746
 - mevalonic aciduria 644
 - Pearson syndrome 401
- ceramide trihexosidase **659**, 662
 - deficiency *see* Fabry disease
- cerebellar ataxia
 - Hartnup disease 541, 542
 - see also* ataxia
- cerebellar atrophy
 - biotinidase deficiency 52
 - CDG type Ie 791
 - L-2-hydroxyglutaric aciduria **86**, 87
 - Kearns–Sayre syndrome 394
 - mevalonic aciduria 644
 - NARP 389, **391**
 - see also* cerebral atrophy
- cerebellar dysarthria, chronic/adult GM₁ gangliosidosis 670
- cerebellum
 - biotinidase deficiency 52
- L-2-hydroxyglutaric aciduria **86**, 87
- isovaleric acidemia 59, **60**
- Menkes disease 549
- mitochondrial DNA depletion 407, **407**
- cerebral atrophy
 - argininemia 223
 - argininosuccinic aciduria 217
 - carbamyl phosphate synthetase
 - deficiency 207
 - carnitine palmitoyl transferase I
 - deficiency 269
 - CDG type If 791
 - CDG type Ik 793
 - citrullinemia 212
 - dihydrofolate reductase deficiency 141
 - galactosemia 419
 - glutaric aciduria type I 64, 66, 67, **68**, **69**, 71
 - Hunter disease 575
 - D-2-hydroxyglutaric aciduria 80
 - hyperammonemic coma 196
 - Krabbe disease 728
 - MELAS 375, **375**
 - Menkes disease 548
 - Pearson syndrome 400
 - pyruvate dehydrogenase complex
 - deficiency 361
 - see also* cerebellar atrophy; cortical atrophy
- cerebral blindness, D-2-hydroxyglutaric aciduria 80
- cerebral calcification 836
 - adrenoleukodystrophy 462
 - biotinidase deficiency 52
 - Kearns–Sayre syndrome 394
 - MELAS 375, **376**
- cerebral creatine deficiency 827–30
 - clinical abnormalities **828**, 828–9, **829**, 829
 - genetics and pathogenesis **827**, 830
 - treatment 830
- cerebral degeneration
 - fucosidosis 740–1
 - Sanfilippo disease 580
 - Tay–Sachs disease 680
 - see also* neurodegeneration
- cerebral edema
 - argininemia 224
 - argininosuccinic aciduria 216
 - galactosemia 418, 421
 - maple syrup urine disease 153, 154, 155
 - medium-chain acyl CoA dehydrogenase
 - deficiency 283, 285
 - ornithine transcarbamylase deficiency 198, 199
- cerebral gliosis, multiple acyl CoA dehydrogenase deficiency 319
- cerebral lactic acidosis, pyruvate dehydrogenase complex deficiency 361
- cerebral palsy
 - Lesch–Nyhan disease 488, 489
 - nonketotic hyperglycinemia 181
 - phenylketonuria 115
- cerebral vascular disease 836
 - Fabry disease 661
 - homocystinuria 148
 - Menkes disease 548
 - see also* stroke-like episodes
- cerebrospinal fluid (CSF)
 - lymphocytosis 836
 - methylmalonic acid concentrations 25

- cerebrospinal fluid (CSF) – *cont.*
 mitochondrial DNA depletion 405
 organic acid analysis 5
- cerebrospinal fluid (CSF) lactate 337, **339**, 342
 MELAS 378, 379
 MERRF disease 384
 mitochondrial DNA polymerase deficiency 405
 NARP 390
 pyruvate dehydrogenase complex deficiency 361
- cerebrospinal fluid (CSF) protein
 elevation 836
 Kearns–Sayre syndrome 393, 394
 Krabbe disease 727, 728
 metachromatic leukodystrophy 762
 multiple sulfatase deficiency 775
- ceruloplasmin levels, Menkes disease 549
- cherry red macular spot 837
 galactosialidosis 754, 755, **755**
 GM₁ gangliosidosis 667, **668**
 GM₂ activator deficiency 694, **695**
 GM₂ gangliosidosis 678
 Niemann–Pick disease 710, **710**, 711
 Sandhoff disease 687, **689**
 Tay–Sachs disease 678, 679–80, **680**
- children, heterozygous familial
 hypercholesterolemia treatment 637
- chitotriosidase 704, 722
- cholestasis, α 1-antitrypsin (AT) deficiency 804
- cholestatic jaundice 837
 I-cell disease 614
 long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 296
 Niemann–Pick type C disease 720
see also jaundice
- cholesterol esterification 721
 impairment *see* Niemann–Pick type C disease
- cholesterol levels, inherited
 hyperlipidemias 650
 heterozygous familial
 hypercholesterolemia 634
 homozygous familial
 hypercholesterolemia 632
 lipoprotein lipase deficiency 652, 653
- cholesterol metabolism disorders
 familial hypercholesterolemia *see* familial hypercholesterolemia
 mevalonic aciduria *see* mevalonic aciduria
- cholesterol-processing abnormality *see* Niemann–Pick type C disease
- cholesteryl ester storage disease 733
 clinical abnormalities 736–7
 genetics and pathogenesis **733**, 737
 treatment 737
- cholestryamine therapy, heterozygous familial
 hypercholesterolemia 637
- chondrodysplasia 837
- chorea, D-2-hydroxyglutaric aciduria 80
- choreoathetosis
 cobalamin C disease 35
 propionic acidemia 10
- chronic adult GM₁ gangliosidosis 670–1, 673
- chronic obstructive pulmonary disease (COPD), α 1-antitrypsin (AT) deficiency 805
- chylomicrons, lipoprotein lipase deficiency 648, 649, 650, 652
- cirrhosis 837
 Andersen disease 426
 α 1-antitrypsin (AT) deficiency 804, 806
- citrullinemia 212
 galactosemia 415
 glycogenosis III 450
 hepatorenal tyrosinemia 173, 175, 176
 long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 297
 lysinuric protein intolerance 237
 mitochondrial DNA depletion 406, **406**
 Niemann–Pick disease 711
 Wolman disease 736
- citrate therapy, von Gierke disease 432
- citrulline **210**
 carbamyl phosphate synthetase deficiency 207
 citrullinemia 212, 214
 multiple acyl CoA dehydrogenase deficiency 320
- citrulline therapy
 carbamyl phosphate synthetase deficiency 207
 lysinuric protein intolerance 238
 ornithine transcarbamylase deficiency 201, 201
- citrullinemia 193, 210–14
 clinical abnormalities 210–12, **211**, **214**
 genetics and pathogenesis **210**, 212–13, 213
 pyruvate carboxylase deficiency 348, 351
 treatment 194, **213**, 213–14
- claw hands
 Hunter disease 574, **574**
 Hurler disease 560, **560**
 Hurler–Scheie disease **567**, 568
 I-cell disease 615–16
 Maroteaux–Lamy disease 598, **599**
 mucopolipidosis III 623, **623**
 multiple sulfatase deficiency 771, **771**
 Scheie disease **566**, 567
- clonus
 ankle *see* ankle clonus
 hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin 125
 Pearson syndrome 401
 Tay–Sachs disease 679
- coagulopathy
 argininosuccinic aciduria 218
 CDG type Ib 788
 CDG type In 793
 CDG type Iq 794
 CDG type IId 794
 CDG type IIh 795
 citrullinemia 212
 congenital disorders of glycosylation, type Ia 784
 hepatorenal tyrosinemia 173, 175
- cobalamin A disease 27, 35
- cobalamin B disease 27, 35
- cobalamin C disease 19, **33**, 33–8, 34
 clinical abnormalities **34**, 34–6, **35**
 genetics and pathogenesis 36, 36–7
 treatment 37–8
- cobalamin D disease 19, 33, 34, 36
- cobalamin E disease 34, 36, 37
- cobalamin F disease 19, 33, 34, 36
- cobalamin G disease 34, 36
- cobalamin transport **33**
- coenzyme Q10 therapy
 Kearns–Sayre syndrome 396
 MELAS 379
- cognitive impairment
 carnitine palmitoyl transferase I deficiency 269
 cobalamin C disease 34, 35
 glutaric aciduria type I 65
 mucopolipidosis III 625
 propionic acidemia 9
see also mental impairment
- cold-induced conditions, carnitine palmitoyl transferase II deficiency, late onset 277
- colobomas
 CDG type Id 790
 CDG type Ii 793
 CDG type Iq 794
- coma
 biotinidase deficiency 50
 carnitine palmitoyl transferase I deficiency 268
 carnitine transporter deficiency 254, 256
 hyperammonemic *see* hyperammonemic coma
 isovaleric acidemia 58
 maple syrup urine disease 153, 154, 156
 3-methylcrotonyl CoA carboxylase deficiency 75
 mitochondrial acetoacetyl-CoA thiolase deficiency 96
- complete heart block, Kearns–Sayre syndrome 393, 394, **394**
- compression fractures, lysinuric protein intolerance 236
- computed tomography (CT)
 adrenals, Wolman disease 735, **735**
 adrenoleukodystrophy 461–2, **462**
 Canavan disease 813, **814**
 ethylmalonic encephalopathy 822, **823**
 glutaric aciduria type I 64, 67, **68**, **69**
 hepatic, hepatorenal tyrosinemia 173
 holocarboxylase synthetase deficiency 42
 homocystinuria 147
 3-hydroxy-3-methylglutaryl CoA lyase deficiency 329
 D-2-hydroxyglutaric aciduria 80
 L-2-hydroxyglutaric aciduria **86**, 87
 lactic acidemias **343**, 343–4
 maple syrup urine disease 155
 MELAS 375, **376**
 methylmalonic acidemia 22, 23
 pyruvate carboxylase deficiency **350**
see also neuroimaging
- congenital disorders of glycosylation (CDG) 787–99, 790
 type I 788, **788**, 790
 type Ia *see* congenital disorders of glycosylation, type Ia
 type Ib 788–9, 790, **791**, 795, 799
 type Ic 789–90, 790, 791, **791**, **792**, 796
 type Id 790, 790, 796
 type Ie 790, 791, **792**, 796
 type If 790, 791–2, 796
 type Ig 790, 792–3, 796
 type Ih 790, 793, 796–7
 type Ii 790, 793, 797
 type Ij 790, 793, 797

- type Ik 790, 793, 797
 type IL 790, 793, 797
 type Im 790, 793, 797
 type In 790, 793, 797
 type Io 790, 793–4, 797–8
 type Ip 790, 794, 798
 type Iq 790, 794, 798
 type II 788, **789**, 790
 type IIa 790, 794, 798
 type IIb 790, 794, 798
 type IIc 790, 794, 798, 799
 type IId 790, 794, 798–9
 type IIE 790, 794, 799
 type IIIf 790, 794–5, 799
 type IIg 790, 795, 799
 type IIh 790, 795, 799
 type Iii 795, 799
 type IIj 790, 795, 799
 congenital disorders of glycosylation, type
 Ia 781–5
 clinical abnormalities 781–4, **782**, **783**, **784**
 genetics and pathogenesis **781**, 784–5, 790
 treatment 785
 congenital heart disease, phenylketonuria 115
 convulsions
 argininemia 223
 carbamyl phosphate synthetase
 deficiency 205, 206
 carnitine palmitoyl transferase I
 deficiency 268
 congenital disorders of glycosylation, type
 Ia 783
 fructose-1,6-diphosphatase deficiency 355
 glutaric aciduria type I 65
 GM₁ gangliosidosis 668
 isovaleric acidemia 58
 maple syrup urine disease 153, 156
 ornithine transcarbamylase deficiency 198
 von Gierke disease 430
 see also seizures
 copper histidinate therapy, Menkes disease 550
 copper levels, Menkes disease 549–50
 copper transport abnormality, Menkes
 disease 546–7, 549, 550
 corneal disease, cystinosis 534, 537
 corneal opacity 837
 Fabry disease 661
 galactosialidosis 754, 755
 GM₁ gangliosidosis 667
 Hurler disease 560, **560**
 Hurler–Scheie disease 567, **569**
 I-cell disease 615
 α -mannosidosis 746
 Maroteaux–Lamy disease 600, 602
 Morquio disease 591, 593
 mucopolipidosis III 624
 multiple sulfatase deficiency 772
 Scheie disease 567
 Sly disease 606, 607
 corneal ulcers
 biotinidase deficiency 49
 cystinosis 534
 oculocutaneous tyrosinemia 165, **165**
 cornstarch supplementation
 3-hydroxy-3-methylglutaryl CoA lyase
 deficiency 331
 glycogenosis type III 452
 mitochondrial DNA depletion 409
 von Gierke disease 434
 coronary artery disease, familial
 hypercholesterolemia 632, 634
 corpus callosum agenesis 837
 D-2-hydroxyglutaric aciduria 81
 cortical atrophy
 CDG type IIc 794
 HHH syndrome 231
 MELAS 375, **375**
 mevalonic aciduria 644
 mitochondrial DNA depletion 408
 neonatal adrenoleukodystrophy 474
 see also cerebral atrophy
 coxa valga, fucosidosis 741
 cranosynostosis, mucopolipidosis III 622, **623**
 cravings, citrullinemia 212
 creatine kinase (CK) 837–8
 carnitine palmitoyl transferase I
 deficiency 269
 carnitine palmitoyl transferase II
 deficiency 274, 277
 carnitine palmitoyl transferase II deficiency,
 late onset 277
 carnitine transporter deficiency 254
 carnitine–acylcarnitine translocase
 deficiency 262–3
 CDG type Ie 791
 CDG type IId 794
 ethylmalonic encephalopathy 823
 fatty acid oxidation disorders 247
 glutaric aciduria type I 67
 glycogenosis type III 450, 453
 long-chain L-3-hydroxyacyl CoA
 dehydrogenase deficiency 296, 297
 medium-chain acyl CoA dehydrogenase
 (MCAD) deficiency 283
 mevalonic aciduria 645
 mitochondrial DNA depletion 404, 407, 409
 Pompe disease 442
 short-chain 3-hydroxyacyl CoA
 dehydrogenase deficiency 309
 very long-chain acyl CoA dehydrogenase
 deficiency 289, 290, 292
 creatine levels, cerebral creatine deficiency 827,
 827
 creatine metabolism 827, **827**
 creatine metabolism disorders 827–30
 clinical abnormalities **828**, 828–9, **829**, 829
 genetics and pathogenesis **827**, 830
 creatine therapy
 arginine:glycine amidinotransferase
 deficiency 830
 cobalamin C disease 37
 guanidinoacetate methyltransferase
 deficiency 830
 creatine transporter (CRTR) deficiency 827,
 827
 clinical abnormalities **828**, 828–9, 829
 genetics and pathogenesis 828, 830
 treatment 830
 cross-reacting material (CRM)
 adenine phosphoribosyl-transferase
 deficiency 499
 argininemia 225
 argininosuccinic aciduria 219
 biotinidase deficiency 52–3
 cholesteryl ester storage disease 737
 citrullinemia 213
 dihydropteridine reductase deficiency 129
 galactosemia 420
 hepatorenal tyrosinemia 174, 175
 homocystinuria 148
 methylmalonic aciduria 26
 ornithine transcarbamylase deficiency 200
 phenylketonuria 115
 Tay–Sachs disease 682
 cryptorchidism, congenital disorders of
 glycosylation 793
 crystalluria
 adenine phosphoribosyl-transferase
 deficiency 499
 hypoxanthine–guanine phosphoribosyl
 transferase deficiency 483–4, 487, **487**,
 488
 orotic aciduria 519
 phosphoribosylpyrophosphate synthetase
 abnormalities 504
 CTNLI gene 210, 212
 CTNS gene 535, 536
 cutaneous lesions *see* skin lesions
 cutaneous xanthomas
 familial hypercholesterolemia 632, **633**, **634**
 lipoprotein lipase deficiency **649**, 651
 Niemann–Pick disease 710
 von Gierke disease 431
 cyanide–nitroprusside test 527, **527**
 cyanosis, Pompe disease 439
 cystathionine synthase 144, **144**, 148
 deficiency *see* homocystinuria
 cysteamine therapy, cystinosis **536**, 536–7
 cystic fibrosis, α 1-antitrypsin (AT)
 deficiency 806
 cystine 525, **525**
 cystine crystals, cystinosis 535, **535**
 cystine excretion
 argininemia 225
 cystinuria 527
 cystine levels, cystinosis 535
 cystine stones, cystinuria **526**, 526–7
 cystinosis 535
 cystinosis 532–7
 clinical abnormalities **533**, 533, 533–5, **534**,
 535
 genetics and pathogenesis **532**, 535–6
 treatment **536**, 536–7
 cystinuria 525–9
 clinical abnormalities **526**, 526–7
 genetics and pathogenesis **527**, 527–8
 treatment 528–9, **529**
 cysts
 renal 844
 subependymal *see* subependymal cysts
 cytoplasmic inclusions
 adrenoleukodystrophy 462, **463**
 GM₁ gangliosidosis 668
 I-cell disease **613**, 617
 Maroteaux–Lamy disease 601
 mucopolipidosis III 625
 neonatal adrenoleukodystrophy 474
 Niemann–Pick type C disease 721
 Sandhoff disease 688
 Tay–Sachs disease 681
 cytosolic acetoacetyl CoA thiolase
 deficiency 100
 dark urine, alkaptonuria 105–6, **106**

- deafness 845
 adenosine deaminase deficiency 508
 Kearns–Sayre syndrome 394
 Krabbe disease 728
 Maroteaux–Lamy disease 600
 MERRF disease 383
 multiple sulfatase deficiency 770
 phosphoribosylpyrophosphate synthetase abnormalities 504
see also hearing loss
- debrancher enzyme (amylo-1,6-glucosidase) 447, **447**, 452
 deficiency *see* glycogenosis type III
- decerebrate posturing
 argininosuccinic aciduria 216
 citrullinemia 211
 Tay–Sachs disease 680
- deep tendon reflexes
 adrenoleukodystrophy 460
 argininemia 223
 biotinidase deficiency 51
 Canavan disease 813
 carnitine transporter deficiency 255
 ethylmalonic encephalopathy 820
 galactosialidosis 755
 glycosylation disorders 783
 GM₁ gangliosidosis 668
 HHH syndrome 230, 231
 hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin 125
 isovaleric acidemia 59
 Krabbe disease 728
 Lesch–Nyhan disease 484
 long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 297
 maple syrup urine disease 153
 metachromatic leukodystrophy 761
 mevalonic aciduria 644
 multiple sulfatase deficiency 770
 NARP 388
 neonatal adrenoleukodystrophy 470
 nonketotic hyperglycinemia 181
 Pearson syndrome 401
 phenylketonuria 115
 Pompe disease 441
 propionic acidemia 10
 pyruvate dehydrogenase complex deficiency 361
 Sanfilippo disease 581
 Wolman disease 736
- dehydration
 3-hydroxy-3-methylglutaryl CoA lyase deficiency 326
 mitochondrial acetoacetyl-CoA thiolase deficiency 96
- dementia
 Canavan disease 813
 cobalamin C disease 34
 MELAS 376
 MERRF disease 383
 metachromatic leukodystrophy 762
 Niemann–Pick type C disease 720
 Sanfilippo disease 583
- demyelination
 adrenoleukodystrophy 462, 464
 GM₂ activator deficiency 695
 Krabbe disease 728
 metachromatic leukodystrophy 763, 764
 NARP 390, 391
 neonatal adrenoleukodystrophy 474
 Tay–Sachs disease 681
see also myelination
- dental anomalies
 Hurler disease 559
 Maroteaux–Lamy disease 601
 Morquio syndrome 591
 mucopolipidosis III 624, **625**
- deoxyadenosine levels, adenosine deaminase deficiency 509
- 1-deoxygalactonojirimycin 663
- depression, homocystinuria 147
- dermatan sulfate
 Hunter disease 576
 Hurler disease **558**, 562
 Maroteaux–Lamy disease 597, **597**, 601
- dermatomyositis, cystinuria **526**
- dermatosis 838
 Hartnup disease 541
 holocarboxylase synthetase deficiency 41
see also skin lesions
- developmental delay
 4-hydroxybutyric aciduria 89
 adenosine deaminase deficiency 508
 arginine:glycine amidinotransferase (AGAT) deficiency 828
 argininemia 223
 aromatic L-amino acid decarboxylase deficiency 137
 CDG type Ig 793
 CDG type Ij 793
 CDG type IL 793
 CDG type In 793
 CDG type IId 794
 creatine transporter deficiency 828
 dihydrofolate reductase deficiency 141
 ethylmalonic encephalopathy 820
 Gaucher disease 701
 glycosylation disorders 782
 guanidinoacetate methyltransferase deficiency 828, 829
 HHH syndrome 230
 Hurler disease 560
 D-2-hydroxyglutaric aciduria 79
 L-2-hydroxyglutaric aciduria 86
 I-cell disease 614–15
 Krabbe disease 728
 Lesch–Nyhan disease 484
 medium-chain acyl CoA dehydrogenase deficiency 283
 MELAS 376
 3-methylcrotonyl CoA carboxylase deficiency 75
 methylmalonic acidemia 20, 21–2
 mevalonic aciduria 643
 mitochondrial acetoacetyl CoA thiolase deficiency 96
 NARP 390
 neonatal adrenoleukodystrophy 470–1
 Niemann–Pick type B disease 711
 Niemann–Pick type C disease 720
 nonketotic hyperglycinemia 182
 phenylketonuria 113
 propionic acidemia 9, 10
 pyruvate dehydrogenase complex deficiency 361
 short-chain acyl CoA dehydrogenase deficiency 304
 Sly disease 606
 Tay–Sachs disease 679
 Wolman disease 736
- dextromethorphan, nonketotic hyperglycinemia management 185
- diabetes mellitus
 erroneous diagnosis 838
 Kearns–Sayre syndrome 394
 MELAS 374, 377
- diarrhea 838
 adenosine deaminase deficiency 508
 CDG type Ib 789
 CDG type Ih 793
 Fabry disease 661
 hepatorenal tyrosinemia 172
 Hunter disease 574–5
 lysinuric protein intolerance 235–6, 238
 3-methylcrotonyl CoA carboxylase deficiency 75
 mevalonic aciduria 643, 644
 Pearson syndrome 399
 Sly disease 606
 von Gierke disease 431
 Wolman disease 733, 736
- dicarboxylic aciduria 7, 247, **249**, 250
 3-hydroxyacyl CoA dehydrogenase deficiency 310
 medium-chain 284, 287
 mitochondrial DNA depletion 406
 neonatal adrenoleukodystrophy 476
 very long-chain acyl CoA dehydrogenase deficiency 289, 290–1
 von Gierke disease 431
 Zellweger syndrome 476
- dichloroacetate (DCA) therapy
 lactic acidemia 344
 MELAS 379
 pyruvate dehydrogenase complex deficiency 364
- dietary protein intolerance
 argininemia 223
 lysinuric protein intolerance *see* lysinuric protein intolerance
 maple syrup urine disease 156
 methylmalonic acidemia 20
 propionic acidemia 9, 14
- dietary therapy
 adenine phosphoribosyl-transferase deficiency 500
 adrenoleukodystrophy 465
 alkaptonuria 109
 argininemia 226, 227
 carbamyl phosphate synthetase deficiency 207
 carnitine palmitoyl transferase I deficiency 271
 carnitine–acylcarnitine translocase deficiency 265
 ethylmalonic encephalopathy 825
 galactosemia 418, 419, 422
 glutaric acidemia I 71
 glycogenosis type III 452–3
 Hartnup disease 542
 heterozygous familial hypercholesterolemia 637
 HHH syndrome 232
 3-hydroxy-3-methylglutaryl CoA lyase

- deficiency 331
 isovaleric acidemia 61
 Lesch–Nyhan disease 494
 lipoprotein lipase deficiency 653–4
 long-chain L-3-hydroxyacyl CoA
 dehydrogenase deficiency 299
 lysinuric protein intolerance 238
 maple syrup urine disease 156, 159
 3-methylcrotonyl CoA carboxylase
 deficiency 77
 methylmalonic acidemia 27
 multiple acyl CoA dehydrogenase
 deficiency 321–2
 oculocutaneous tyrosinemia **168**, 168–9
 phenylketonuria 118–19
 propionic acidemia 14–15
 pyruvate dehydrogenase complex
 deficiency 364
 short/branched chain acyl CoA
 dehydrogenase deficiency 314
 dihydrofolate reductase (DHFR) 141–2
 dihydrofolate reductase (DHFR)
 deficiency 141–2
 dihydropteridine reductase (DHPR) **123**, 124
 dihydropteridine reductase (DHPR)
 deficiency 125, 128, 128, 129, 130
 2,8-dihydroxyadenine 499, 500
 DIMPLES mnemonic 3
 dislocated lens 838
 homocystinuria 145, **145**, 150
 diverticula, Fabry disease 661
 D-lactic acid 6–7
 docosahexanoic acid (DHA) levels,
 peroxisomal biogenesis disorders 476
 docosahexanoic acid (DHA) supplementation,
 long-chain L-3-hydroxyacyl CoA
 dehydrogenase deficiency 299
 dolichol 748
 L-DOPA therapy
 aromatic L-amino acid decarboxylase
 deficiency 139
 hyperphenylalaninemia and defective
 metabolism of tetrahydrobiopterin 131
 tyrosine hydroxylase deficiency 139, 140
 dopamine, Lesch–Nyhan disease 493–4
 DOPA-responsive dystonias 140
 drooling
 argininemia 223
 CDG type Ie 791
 hyperphenylalaninemia and defective
 metabolism of tetrahydrobiopterin 125
 Sanfilippo disease 581
 drugs, carbohydrate content 357
 Duarte variant 419–20, 422
 dwarfism
 CDG type IIc 794
 see also short stature
 dysarthria
 adrenoleukodystrophy 460
 Canavan disease 813
 chronic/adult GM1 gangliosidosis 670
 L-2-hydroxyglutaric aciduria 86
 Lesch–Nyhan disease 485
 metachromatic leukodystrophy 761, 762
 pyruvate carboxylase deficiency 350
 Sandhoff disease 688
 see also speech problems
 dysmetria
 L-2-hydroxyglutaric aciduria 86
 isovaleric acidemia 59
 Kearns–Sayre syndrome 394
 dysmorphic features
 carnitine palmitoyl transferase II
 deficiency 274–5, 275
 CDG type Ig 793
 CDG type Ih 793
 CDG type Ij 793
 CDG type Ik 793
 CDG type Ilb 794
 CDG type Ile 794
 glycogenosis type III 450
 D-2-hydroxyglutaric aciduria 80
 mevalonic aciduria 644, **644**
 multiple acyl CoA dehydrogenase
 deficiency 317, **318**, 319
 neonatal adrenoleukodystrophy **471**, 471–2,
 472
 phenylketonuria **113**, 115
 pyruvate dehydrogenase complex
 deficiency 361, **361**, 362
 see also facial features
 dysostosis multiplex 557, 838
 fucosidosis 741
 galactosialidosis 754
 GM1 gangliosidosis 668, **668**, **669**
 Hunter disease **574**, **575**, 576
 Hurler disease 560–1, **561**, **562**
 I-cell disease **616**, 617, **617**
 α -mannosidosis 745–6, **747**
 Maroteaux–Lamy disease **600**, 601, **601**
 mucopolipidosis III **621**, 621–2, **622**
 multiple sulfatase deficiency 771, 773, **774**
 Sanfilippo disease 583–4, **584**
 Sly disease 607
 dysphagia
 adrenoleukodystrophy 460
 argininemia 223
 hyperphenylalaninemia and defective
 metabolism of tetrahydrobiopterin 125
 metachromatic leukodystrophy 761
 Niemann–Pick type C disease 720
 dyspnea
 α 1-antitrypsin (AT) deficiency 805
 Fabry disease 661
 multiple acyl CoA dehydrogenase
 deficiency 317
 dystonia 5
 aromatic L-amino acid decarboxylase
 deficiency 137
 chronic/adult GM1 gangliosidosis 670
 creatine transporter deficiency 829
 glutaric aciduria type I 65, 66, **66**, **67**, 70
 guanidinoacetate methyltransferase
 deficiency 828
 homocystinuria 147, 148
 Lesch–Nyhan disease 484, **484**
 methylmalonic acidemia 22
 Niemann–Pick type C disease 719
 propionic acidemia 10
 tyrosine hydroxylase deficiency 139, 140
 dystonic posturing, propionic acidemia 10
 dystrophic nails, lysinuric protein
 intolerance 236
 early infantile galactosialidosis 753–4, 755,
 756
 ecchymoses, ethylmalonic encephalopathy 821,
 821
 ECG *see* electrocardiogram (ECG)
 ectopia lentis 838
 homocystinuria 145, **145**, 150
 eczematoid rash, phenylketonuria 113, **113**
 edema
 CDG type Ih 793
 CDG type Ilb 794
 ethylmalonic encephalopathy 821
 galactosialidosis 753
 GM1 gangliosidosis 667
 see also cerebral edema
 EEG *see* electroencephalogram (EEG)
 Ehlers–Danlos syndrome type IX 550
 electrocardiogram (ECG)
 α 1-antitrypsin (AT) deficiency 805
 carnitine transporter deficiency 254
 glycogenosis type III 450
 Kearns–Sayre syndrome 394, **394**
 α -mannosidosis 747
 Pompe disease 439–40, **441**
 electroencephalogram (EEG) 838
 adrenoleukodystrophy 462
 argininemia 223
 argininosuccinic aciduria 216, 217
 aromatic L-amino acid decarboxylase
 (AADC) deficiency 139
 biotinidase deficiency 52
 Canavan disease 813–14
 carnitine palmitoyl transferase I
 deficiency 269
 cobalamin C disease 35
 dihydrofolate reductase deficiency 141
 ethylmalonic encephalopathy 822
 fructose-1,6-diphosphatase deficiency
 355
 Gaucher disease, type III 702
 glutamine synthetase deficiency 242
 glutaric aciduria 65
 GM1 gangliosidosis, infantile 668
 GM1 gangliosidosis, late infantile/
 juvenile 669
 guanidinoacetate methyltransferase
 deficiency 828
 holocarboxylase synthetase (HCS)
 deficiency 42, 44
 4-hydroxybutyric aciduria 89, 92
 D-2-hydroxyglutaric aciduria 80
 L-2-hydroxyglutaric aciduria 87
 hyperphenylalaninemia 126
 isovaleric acidemia 59
 Krabbe disease 728
 lactic acidemia 370
 maple syrup urine disease 155
 MELAS 377
 Menkes disease 548
 MERRF disease 383–4
 metachromatic leukodystrophy 762
 methylmalonic acidemia 23
 mevalonic aciduria 644
 neonatal adrenoleukodystrophy 470
 nonketotic hyperglycinemia 181, 183
 phenylketonuria 115, 119
 propionic acidemia 10
 pyruvate carboxylase deficiency 350
 Sandhoff disease 687
 Tay–Sachs disease 680

- electromyogram (EMG)
 glycogenosis III 450
 Kearns–Sayre syndrome 394
 Krabbe disease 728
 MELAS 375
 Pompe disease 442
 electron transfer flavoprotein (ETF) 317, **317**
 deficiency 316–17, 320–1
 electron transport chain **340, 341, 341**
 disorders 340–2, 344
 EMG *see* electromyogram (EMG)
 emphysema, α 1-antitrypsin (AT)
 deficiency 803, 805, 807
 encephalitis 835
 encephalomyopathy, MELAS 374, 376, 378
 encephalopathic episodes, glutaric aciduria
 type I 64–5, 67, 70, 71
 encephalopathy
 ethylmalonic *see* ethylmalonic
 encephalopathy
 glutamine synthetase deficiency 241
 medium-chain acyl CoA dehydrogenase
 deficiency 283, 285
 mitochondrial DNA depletion 404, 406,
 408, 409
 short-chain 3-hydroxyacyl CoA
 dehydrogenase deficiency 309
 endocardial fibroelastosis, Hurler disease 560
 enteropathy
 CDG type Ib 788
 CDG type Ih 793
 enzyme replacement therapy
 adenosine deaminase deficiency 510
 α 1-antitrypsin (AT) deficiency 807
 Gaucher disease 704
 Hurler disease 563–4
 metachromatic leukodystrophy 764
 Morquio syndrome 594
 multiple sulfatase deficiency 776
 Pompe disease 444
 Sanfilippo disease 585
 epilepsy partialis continua (EPC) 406
 epilepsy
 CDG type Ic 789
 CDG type Id 790
 CDG type Ie 791
 CDG type IIa 794
 see also convulsions; seizures
 epinephrine, response in von Gierke
 disease 431
 erythrocyte sedimentation rate 843
 erythroderma, CDG type If 791
 erythrophagocytosis 839
 lysinuric protein intolerance 237
ETHE1 gene 824, 825
 ethylhydracrylic acid 824
 ethylmalonic acid **819, 824**
 multiple acyl CoA dehydrogenase
 deficiency 316, 320
 short-chain acyl CoA dehydrogenase
 deficiency 302, 303, 304
 ethylmalonic aciduria 303, 820, 823
 ethylmalonic encephalopathy 819–25
 clinical abnormalities **820, 820–3, 821, 822,**
 823, 824
 genetics and pathogenesis **819, 823–5, 825**
 treatment 825
 ethylmalonic–adipic aciduria 319, 820
 see also multiple acyl CoA dehydrogenase
 deficiency (MADD)
 exercise intolerance 838–9
 Kearns–Sayre syndrome 394
 MELAS 374, 375
 MERRF disease 383
 multiple acyl CoA dehydrogenase
 deficiency 319
 pyruvate dehydrogenase complex
 deficiency 363
 exercise-induced conditions, carnitine
 palmitoyl transferase II deficiency, late
 onset 277
 extracorporeal membrane oxygenation
 (ECMO) 194
 ornithine transcarbamylase deficiency 201
 extrapyramidal movements, isovaleric
 acidemia 59
 ezetimibe therapy, heterozygous familial
 hypercholesterolemia 637
 Fabry disease 659–63, 668, 669
 clinical abnormalities 659, 659–61, **660**
 genetics and pathogenesis **659, 661–2, 662,**
 679
 treatment 662–3
 facial features
 carnitine palmitoyl transferase II
 deficiency 274, **275**
 CDG type Ia 782, **784**
 CDG type Ic **791**
 CDG type Ig 793
 CDG type Ih 793
 CDG type Ik 793
 CDG type IIb 794
 ethylmalonic encephalopathy 821, 822, **822**
 fucosidosis 741, **741**
 galactosialidosis **754, 755**
 glutamine synthetase deficiency 241
 glycogenosis type III 450
 GM₁ gangliosidosis, infantile 667, **667**
 GM₁ gangliosidosis, late infantile/
 juvenile 669, **670, 671**
 Hunter disease **573, 574, 575**
 Hurler disease 558–60, **559, 560**
 Hurler–Scheie disease 567, **568, 569**
 D-2-hydroxyglutaric aciduria 80
 I-cell disease 615, **615**
 α -mannosidosis 745, **746**
 Maroteaux–Lamy disease 597, **598**
 Menkes disease 548
 methylmalonic acidemia 21, **22**
 mevalonic aciduria 644, **644, 646**
 Morquio syndrome **589, 590, 591**
 mucopolidosis III 623–4, **624**
 multiple acyl CoA dehydrogenase
 deficiency 317, **318**
 multiple sulfatase deficiency **770, 771, 771,**
 772
 neonatal adrenoleukodystrophy **471, 472,**
 472
 Niemann–Pick disease 711, **712**
 phenylketonuria **113, 115**
 propionic acidemia 11, **12**
 pyruvate dehydrogenase complex
 deficiency 361, **361, 362**
 Sandhoff disease 687, **687, 688, 689**
 Sanfilippo disease **582, 583, 583**
 Scheie disease 567, **567**
 Sly disease 606, **606**
 Tay–Sachs disease **680, 680**
 FAF1 (peroxisomal assembly factor 1) 469
 failure to thrive 839
 α 1-antitrypsin (AT) deficiency 804
 argininemia 223
 CDG type Ie 791
 CDG type Ig 793
 CDG type IIg 795
 cobalamin C disease 34
 congenital disorders of glycosylation, type
 Ia 782
 galactosemia 415, **416**
 GM₁ gangliosidosis 667
 hepatorenal tyrosinemia 172
 HHH syndrome 230
 holocarboxylase synthetase deficiency 42
 hyperphenylalaninemia and defective
 metabolism of tetrahydrobiopterin 125
 lysinuric protein intolerance 235, **236, 238**
 3-methylcrotonyl CoA carboxylase
 deficiency 75
 methylmalonic acidemia 20, **21**
 mevalonic aciduria 643, **643**
 neonatal adrenoleukodystrophy 470
 Niemann–Pick type A disease 710
 Niemann–Pick type B disease 711
 orotic aciduria 518
 Pompe disease 439, 441
 severe combined immunodeficiency
 disease 508
 short-chain 3-hydroxyacyl CoA
 dehydrogenase deficiency 309
 short-chain acyl CoA dehydrogenase
 deficiency 304
 familial amaurotic idiocy 678
 familial defective apolipoprotein B-100
 (FDB) 631, 634–5
 familial hypercholesterolemia 631–7, **650**
 clinical abnormalities 632–4, **633, 634**
 genetics and pathogenesis 634–6
 treatment 636–7
 Fanconi syndrome 533, 845
 cystinosis 533, 535
 galactosemia 418
 hepatorenal tyrosinemia 171, 173, 175, 176
 lysinuric protein intolerance 237
 MELAS 377
 von Gierke disease 431, 432
 Fanconi–Bickel syndrome 427, 433
 fasting
 carnitine palmitoyl transferase II deficiency,
 late onset 277, 278
 carnitine transporter deficiency 254, 255
 carnitine–acylcarnitine translocase
 deficiency 261
 medium-chain acyl CoA dehydrogenase
 deficiency 282, **283, 284, 286**
 propionic acidemia 15
 fat pads, congenital disorders of glycosylation,
 type Ia 782, **783, 784**
 fatigue
 cobalamin C disease 34
 MELAS 375
 fatty acid oxidation **248**
 fatty acid oxidation disorders 6, 192–3, 247–51
 carnitine palmitoyl transferase I deficiency

- see carnitine palmitoyl transferase (CPT) I deficiency
 carnitine palmitoyl transferase II deficiency
 see carnitine palmitoyl transferase II deficiency
 carnitine transporter deficiency *see* carnitine transporter deficiency (CTD)
 carnitine-acylcarnitine translocase deficiency *see* carnitine-acylcarnitine translocase deficiency
 clinical abnormalities 247, 338
 diagnostic work-up 247, **249**
 3-hydroxy-3-methylglutaryl CoA lyase deficiency *see* 3-hydroxy-3-methylglutaryl (HMG) CoA lyase deficiency
 long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency *see*
 long-chain L-3-hydroxyacyl CoA dehydrogenase (LCHAD) deficiency
 medium-chain acyl CoA dehydrogenase deficiency *see* medium-chain acyl CoA dehydrogenase (MCAD) deficiency
 multiple acyl CoA dehydrogenase deficiency *see* multiple acyl CoA dehydrogenase deficiency
 short/branched chain acyl CoA dehydrogenase (SBCAD) deficiency 312–14, **313**
 short-chain 3-hydroxyacyl CoA dehydrogenase deficiency **309**, 309–10
 short-chain acyl CoA dehydrogenase deficiency *see* short-chain acyl CoA dehydrogenase deficiency
 very long-chain acyl CoA dehydrogenase deficiency *see* very long-chain acyl CoA dehydrogenase (VLCAD) deficiency
 FDP (fructose-1,6-diphosphatase) 354, **354**, 356
 FDP deficiency *see* fructose-1,6-diphosphatase (FDP) deficiency
 fertility, male, phenylketonuria 120
 fetal ascites, Niemann–Pick type C disease 720
 fetal hydrops *see* hydrops fetalis
 α -fetoprotein, hepatorenal tyrosinemia 173, 175, 176
 fever
 glutaric aciduria type I 65, 66
 Krabbe disease 728
 mevalonic aciduria 643, 644–5
 fever syndromes 839
 fibrate therapy, carnitine palmitoyl transferase II deficiency, late onset 279
 flaccidity
 Krabbe disease 728
 maple syrup urine disease 153, **153**
 flat feet, Morquio disease 589
 flexion contractures, GM₁ gangliosidosis 668
 fluoriodoauracil (FIAU) toxicity, mitochondrial DNA depletion 409
 foam cells
 fucosidosis 742, **742**
 galactosialidosis 755
 α -mannosidosis 747
 Niemann–Pick disease 708, 711, 712, 713, **713**, **714**
 Niemann–Pick type C disease 720, 721
 Wolman disease 735, 736, **736**
 folate metabolism/transport disorders 838
 dihydrofolate reductase (DHFR) deficiency 141–2
 methionine synthase deficiency 33, **33**, 36
 methylene tetrahydrofolate reductase deficiency 34, 548
 folinic acid therapy
 aromatic L-amino acid decarboxylase deficiency 139
 dihydrofolate reductase deficiency 141, 142
 dihydropteridine reductase deficiency 131
 formylglycine-generating enzyme (FGE) 769, 770, 776
 fractures 843
 Gaucher disease 698, 700
 lysine protein intolerance 236
 Menkes disease 548
 French pyruvate carboxylase deficiency 348
 frontal bossing, propionic acidemia 11
 frontotemporal atrophy
 glutaric aciduria type I 64, 66, 67, **68**, **69**, 71
 see also cerebral atrophy
 fructose loading
 fructose-1,6-diphosphatase deficiency 356
 lactic acidemias 340
 fructose-1,6-diphosphatase (FDP) 354, **354**, 356
 fructose-1,6-diphosphatase (FDP) deficiency 354–7
 clinical abnormalities **355**, 355–6
 genetics and pathogenesis **354**, 356
 treatment 357, 357
 FUCAI gene 740, 742
 fucose 740, **740**
 fucose supplementation, CDG type IIc 797
 fucosidosis 740–2, **741**, **742**
 fumarylacetoacetate, hepatorenal tyrosinemia 172, 175
 fumarylacetoacetate hydrolase **172**, 174
 fumarylacetoacetate hydrolase deficiency *see* hepatorenal tyrosinemia
 GAA *see* guanidinoacetate (GAA)
 GABA (γ -aminobutyric acid)
 glutaric aciduria type I 72
 4-hydroxybutyric aciduria 89, **90**, 92
 D-2-hydroxyglutaric aciduria 82, 83
 phenylketonuria 116
 gait abnormalities
 adrenoleukodystrophy 460
 cystinosis 534
 α -mannosidosis 745
 metachromatic leukodystrophy 761, 762
 galactitol 418, 419, 421, 422
 galactocerebroside **726**, 729
 galactose 415, 421
 galactose-1-phosphate uridyl transferase 415, **416**, 419, 420
 galactosemia 5, 415–22
 clinical abnormalities 415–19, **416**, **417**
 genetics and pathogenesis 419–22, 421
 treatment 420, 422
 galactosialidosis 752–6
 clinical abnormalities **753**, 753–5, **754**, **755**
 genetics and pathogenesis 755–6
 treatment 756
 β -galactosidase 666, **666**, 672, 752, 755
 α -galactosidase A **659**, 662
 deficiency *see* Fabry disease
 β -galactosidase deficiency
 galactosialidosis 752, 753, 756
 GM₁ gangliosidosis *see* GM₁ gangliosidosis
 Hunter disease 576
 Morquio syndrome type B *see* Morquio B disease
 galactosuria 415
 galactosylceramidase 726, **726**, 729
 galactosylceramide lipidosis *see* Krabbe disease
 GALT gene 729
 gamma glutamyl transferase (GGT) levels, mitochondrial DNA depletion 404, 405, 406
 GAMT deficiency *see* guanidinoacetate methyltransferase (GAMT) deficiency
 gangliosides, Sanfilippo disease 584
 gangliosidoses
 GM₁ *see* GM₁ gangliosidosis
 GM₂ *see* GM₂ gangliosidosis
 gas chromatography–mass spectrometry (GCMS)
 argininemia 225
 Canavan disease 815, 816
 galactosemia 419, 421
 glutaric aciduria type I 70
 holocarboxylase synthetase deficiency 43
 3-hydroxy-3-methylglutaryl CoA lyase deficiency 330, 331
 4-hydroxybutyric aciduria 92
 D-2-hydroxyglutaric aciduria 79, 81–2, 87
 isovaleric acidemia 59
 long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 297
 maple syrup urine disease 158
 medium-chain acyl CoA dehydrogenase deficiency 284, 286
 3-methylcrotonyl CoA carboxylase deficiency 76
 methylmalonic acidemia 25–6
 methylmalonic aciduria and homocystinuria 37
 mevalonic aciduria 642, 643
 organic acid analysis 3, 5
 propionic acidemia 13
 short/branched chain acyl CoA dehydrogenase deficiency 314
 gastrointestinal bleeding
 α 1-antitrypsin (AT) deficiency 804
 hepatorenal tyrosinemia 172, 173
 lipoprotein lipase deficiency 651
 Gaucher cells 698, 700, 702, **703**
 Gaucher disease 698–705
 clinical abnormalities 698, **699**, 699–702, **700**, **701**, **702**
 diagnosis 702, **703**
 genetics and pathogenesis **679**, **699**, 703, 703–4
 treatment 704–5
 type I 698, 698, **699**, 699–702, **700**, **701**, **702**
 type II 698, 698, 702
 type III 698, 698, 702
 gene therapy
 adenosine deaminase deficiency 511
 adrenoleukodystrophy 464
 homozygous familial hypercholesterolemia 637
 Morquio syndrome 594

- gene therapy – *cont.*
 multiple sulfatase deficiency 776–7
 Niemann–Pick type C disease 722
- genu valgum
 homocystinuria 146, **146**, 150
 Maroteaux–Lamy disease 598
 Morquio disease 589, **590**, 591
 Scheie disease **566**, 567
- gibbus
 Hurler disease **559**, 560, 561, **562**
 α -mannosidosis 746
 Sly disease 606
- gingival hyperplasia
 I-cell disease 615, **615**
 mucopolipidosis III 625, **626**
 multiple sulfatase deficiency 772, 773
 Sly disease 606
- glaucoma
 homocystinuria 145, **145**
 oculocutaneous tyrosinemia 165
- globoid cell leukodystrophy *see* Krabbe disease
- globoid cells, Krabbe disease 726, **728**, 729
- globoside 689–90
- glomerulonephritis, α 1-antitrypsin (AT)
 deficiency 806
- glossitis, biotinidase deficiency 50
- glucagon test, von Gierke disease 431
- β -glucocerebrosidase 698, **699**, 703
- glucocerebroside (glycosylceramide) 698, **699**, 704
- glucose-6-phosphatase **428**, 429, 433
 deficiency *see* von Gierke disease
- glucose-6-phosphate transport protein 433
 deficiency 426, 433
- β -glucuronidase 605, **605**, 607
- β -glucuronidase deficiency *see* Sly disease
- glutamine levels
 argininemia 225, 226
 argininosuccinic aciduria 219, 220
 carbamyl phosphate synthetase
 deficiency 207
- citrullinemia 212
- glutamine synthetase deficiency 241, 242–3
 HHH syndrome 231
- hyperammonemia 193
- lysineric protein intolerance 237
- ornithine transcarbamylase deficiency 198, 199
- pyruvate carboxylase deficiency 351
- glutamine synthetase **241**, 243
- glutamine synthetase deficiency 241–3
 clinical abnormalities 241–3, **242**, **243**
 genetics and pathogenesis 243
 treatment 243
- glutamine therapy, pyruvate carboxylase
 deficiency 352
- glutaric acid **65**, 70
 multiple acyl CoA dehydrogenase
 deficiency 316, 320
- glutaric aciduria, type I 64–72, **65**
 clinical abnormalities 64–9, **66**, **67**, **68**, **69**
 genetics and pathogenesis 69–70
 treatment 71, 71–2
- glutaric aciduria, type II *see* multiple acyl CoA
 dehydrogenase deficiency (MADD)
- glutaryl CoA dehydrogenase 64, **65**, 69–70
 deficiency *see* glutaric aciduria, type I
- glycerol loading
 fructose-1,6-diphosphatase deficiency 356
 von Gierke disease 431
- glycine cleavage system 180, **180**, 183
- glycine encephalopathy *see* nonketotic
 hyperglycinemia
- glycine levels
 lysineric protein intolerance 237
 mitochondrial DNA depletion 408
 nonketotic hyperglycinemia 180, 183, **184**,
 184–5
- glycine supplementation
 isovaleric acidemia 61
 multiple acyl CoA dehydrogenase
 deficiency 322
- glycogen **425**, 425–6
- glycogen storage diseases 425–7, **426**
 type I *see* von Gierke disease
 type Ib 428, 432, 433
 type Ic 426, 428, 432
 type II *see* Pompe disease
 type III *see* glycogenosis type III
 type IV 426, 426
 type V 426, 426
- glycogen synthase deficiency 427, **427**
- glycogenesis type I *see* von Gierke disease
- glycogenosis type Ib 428, 432, 433
- glycogenosis type Ic 426, 428, 432
- glycogenosis type II *see* Pompe disease
- glycogenosis type III 447–53
 clinical abnormalities **448**, 448–51, **449**
 genetics and pathogenesis **447**, 451–2
 glycogenosis type I vs. 451, 451
 treatment 452–3
- glycogenosis type IV 426, 426
- glycogenosis type V 426, 426
- glycosaminoglycan excretion, Sly disease 607
- glycosphingolipids, Fabry disease 661–2
- glycosuria 839
 cystinosis 532
 hepatorenal tyrosinemia 173
- glycosylation 787
 congenital disorders *see* congenital disorders
 of glycosylation (CDG)
- GM₁ gangliosidosis 666–74
 clinical abnormalities **667**, 667–71, **668**,
669, **670**, **671**
 genetics and pathogenesis **666**, 671–4, **672**,
 673
 treatment 674
- GM₂ activator 694
- GM₂ activator deficiency 678, 679, 681, 694–6
 clinical abnormalities **694**, 694–5, **695**
 genetics and pathogenesis 695–6
 treatment 696
- GM₂ gangliosidosis 678, 679
 AB variant *see* GM₂ activator deficiency
- adult 679, 681
- juvenile 679, 681
- Sandhoff disease *see* Sandhoff disease
- Tay–Sachs disease *see* Tay–Sachs disease
- GNPTAB gene 626
- gonadal insufficiency,
 adrenoleukodystrophy 461
- gout
 hypoxanthine–guanine phosphoribosyl
 transferase deficiency 483, **487**, 487–8,
 493
 phosphoribosylpyrophosphate synthetase
 abnormalities 504
 von Gierke disease 431, 432
- gouty arthritis
 hypoxanthine–guanine phosphoribosyl
 transferase deficiency 483, 488, 489,
 494
 phosphoribosylpyrophosphate synthetase
 abnormalities 504
- grimacing, glutaric aciduria type I 65, **66**, **68**
- growth
 cystinosis 533, 535, 536
 Gaucher disease 701, **701**
- growth hormone therapy
 MELAS 374
- methylmalonic acidemia 28
- Pearson syndrome 401
- propionic acidemia 15
- GTP cyclohydrolase 124, **124**
- GTP cyclohydrolase deficiency 124, 125, 128,
 128, 129
 diagnosis 130
 treatment 130, **130**
- guanidinoacetate (GAA) 827
 cerebral creatine deficiency 827, **827**, 828,
 829, 829, 830
- guanidinoacetate methyltransferase (GAMT)
 deficiency **827**, 827–8
 clinical abnormalities 828, 829, 829
 genetics and pathogenesis 828, 830
 treatment 828
- Guthrie test 116–17
- gyrate atrophy, hyperornithinemia 230, 231
- hair abnormalities 839
 argininemia 225
 argininosuccinic aciduria 216, 217, **217**, **218**
 Menkes disease **546**, 547, **547**, **547**, **548**
see also alopecia
- hallucinations, visual 846
- hands, claw *see* claw hands
- Hartnup disease **540**, 540–2, **541**, **542**
- HDL levels *see* high density lipoprotein (HDL)
 levels
- headaches
 argininosuccinic aciduria 217
- citrullinemia 212
- MELAS 375–6, 378
- mitochondrial acetoacetyl-CoA thiolase
 deficiency 97
- ornithine transcarbamylase deficiency 199
- hearing loss
 adrenoleukodystrophy 460
- biotinidase deficiency 48, 51, 53
- fucosidosis 741
- galactosialidosis 754
- Hunter disease 574, 575
- α -mannosidosis 746
- β -mannosidosis 749
- MELAS 377
- multiple sulfatase deficiency 770
- neonatal adrenoleukodystrophy 472
- Sanfilippo disease 583
see also deafness
- heart disease *see* cardiac disease
- hematuria
 ethylmalonic encephalopathy 821
 hypoxanthine–guanine phosphoribosyl
 transferase deficiency 488

- Lesch–Nyhan disease 484, 487
 hemianopsia, adrenoleukodystrophy 460
 hemiparesis, 3-hydroxy-3-methylglutaryl CoA lyase deficiency 326
 hemiplegia
 congenital disorders of glycosylation, type Ia 783
 Fabry disease 661
 homocystinuria 147
 hemodialysis
 Fabry disease 662
 hyperammonemia 194, 194
 maple syrup urine disease 158
 hemolytic anemia 839
 hemophagocytosis *see* erythrophagocytosis
 hemorrhages
 argininosuccinic aciduria 217
 intracranial, D-2-hydroxyglutaric aciduria 81
 propionic acidemia 9
 retinal, glutaric aciduria type I 68
 see also bleeding
 hemorrhagic streaks, ethylmalonic encephalopathy 821, **821**
 heparan sulfate
 Hunter disease 576
 Hurler disease 558, 562
 Sanfilippo disease 580, **581**, 584, 585
 heparan-N-sulfatase, defective activity 580, **581**, 585
 see also Sanfilippo disease
 hepatic adenomas, von Gierke disease 429, 431, 432, 433, 434
 hepatic carcinoma 839
 α 1-antitrypsin (AT) deficiency 805
 hepatorenal tyrosinemia 173, 176
 von Gierke disease 432
 hepatic cirrhosis *see* cirrhosis
 hepatic disease
 α 1-antitrypsin (AT) deficiency 803–4, **805**, 805–6, **806**, 807
 see also specific liver diseases
 hepatic failure 839
 CDG type Ib 788, 789
 citrullinemia 212
 hepatorenal tyrosinemia 171–2, 176
 LCHAD deficiency 251
 hepatic fibrosis
 argininosuccinic aciduria 218
 CDG type Ib 788, 789
 neonatal adrenoleukodystrophy 474
 hepatic insufficiency
 lysine protein intolerance 237
 Pearson syndrome 401
 hepatic necrosis, long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 295, 296
 hepatic phosphorylase deficiency 426, 426
 hepatic presentations, neonatal 842
 hepatic steatosis
 carnitine palmitoyl transferase I deficiency 269
 carnitine–acylcarnitine translocase deficiency 262, **262**
 congenital disorders of glycosylation, type Ia 784
 hepatic transplantation *see* liver transplantation
 hepatitis
 adenosine deaminase deficiency 508
 α 1-antitrypsin (AT) deficiency 804
 hepatocellular carcinoma *see* hepatic carcinoma
 hepatocellular damage, hepatorenal tyrosinemia 173, 175
 hepatocytes, α 1-antitrypsin (AT) deficiency 805, **805**
 hepatomegaly
 α 1-antitrypsin (AT) deficiency 804
 argininosuccinic aciduria 216, 218
 carnitine palmitoyl transferase I deficiency 269
 carnitine palmitoyl transferase II deficiency, lethal neonatal 273
 carnitine transporter deficiency 254
 carnitine–acylcarnitine translocase deficiency 262
 CDG type IL 793
 CDG type In 793
 CDG type IIb 794
 citrullinemia 211
 congenital disorders of glycosylation, type Ia 782
 fructose-1,6-diphosphatase deficiency 355, 357
 galactosemia 415, **416**, 419
 Gaucher disease 698, 699
 glutaric aciduria type I 66
 glycogenosis type III 448, **449**, 450
 hepatorenal tyrosinemia 172
 3-hydroxy-3-methylglutaryl CoA lyase deficiency 326
 long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 296
 lysine protein intolerance 236, 238
 Maroteaux–Lamy disease 599
 medium-chain acyl CoA dehydrogenase deficiency 283
 methylmalonic acidemia 23
 neonatal adrenoleukodystrophy 470–1
 ornithine transcarbamylase deficiency 199
 Pompe disease 439
 pyruvate carboxylase deficiency 348
 von Gierke disease 429, 431
 hepatorenal tyrosinemia 5, 171–6
 clinical abnormalities 171–4, **172**, **173**, **174**
 genetics and pathogenesis **172**, 174, 174–6, **175**
 treatment 176, **176**
 hepatosplenomegaly
 CDG type IIe 794
 CDG type IIg 795
 CDG type IIk 793
 cholesteryl ester storage disease 736
 fucosidosis 741
 galactosialidosis 753, 754
 GM₁ gangliosidosis 667–8
 Hurler disease 560, 564
 Hurler–Scheie disease **568**
 lipoprotein lipase deficiency 650
 α -mannosidosis 745
 Maroteaux–Lamy disease 599
 mevalonic aciduria 643
 multiple sulfatase deficiency 771, **771**, 772
 Niemann–Pick disease 708, 709, **709**, 710, **710**, 711
 Niemann–Pick type C disease **719**, 720
 Sandhoff disease 686, 688
 Sanfilippo disease 583
 Sly disease 606
 Wolman disease 734, 735
 hereditary tyrosinemia *see* hepatorenal tyrosinemia
 hernias
 Hunter disease 574
 Hurler disease 558, 560, 564
 I-cell disease 616
 α -mannosidosis 745
 Maroteaux–Lamy disease 597
 Morquio syndrome 591
 mucopolidosis III 625
 Sanfilippo disease 583
 Sly disease 606
 heterozygous familial
 hypercholesterolemia 631, 632
 clinical abnormalities 634
 genetics and pathogenesis 635, 636
 treatment 637
 hexacosanoic acid
 adrenoleukodystrophy 463
 neonatal adrenoleukodystrophy 474
 hex-B subunit deficiency *see* Sandhoff disease
 hexosaminidase A 678, **679**, 686, 690
 hexosaminidase A and B deficiency *see* Sandhoff disease
 hexosaminidase A deficiency 679
 adult GM₂ gangliosidosis 679, 681
 juvenile GM₂ gangliosidosis 679, 681
 Tay–Sachs disease 678, 681, 682, 682
 hexosaminidase B 678, 686, 690
 HHH (hyperornithinemia, hyperammonemia, homocitrullinuria) syndrome 191, 229–33
 clinical abnormalities **230**, 230–1, 231
 genetics and pathogenesis **229**
 treatment 232–3
 hiccuping, nonketotic hyperglycinemia 181
 HIDS (hyperimmunoglobulin D and periodic fever syndrome) 643, 645, 646
 high density lipoprotein (HDL) levels
 elevated
 carnitine palmitoyl transferase I deficiency 271
 familial hypercholesterolemia 634
 see also hyperlipidemia
 low 839
 hirsutism
 Maroteaux–Lamy disease 597
 mucopolidosis III 624
 multiple sulfatase deficiency 771, **771**
 histidine levels, histidinuria 545, **545**
 histidinuria **544**, 544–5, **545**
 histiocytosis, GM₁ gangliosidosis 668–9, **669**
 HMG CoA reductase inhibitors *see* statin therapy
 holocarboxylase synthetase (HCS) **40**, **40**
 holocarboxylase synthetase (HCS) deficiency 5, 40–4
 clinical abnormalities 41–2, **42**
 genetics and pathogenesis 42–3, 43
 treatment 44, **44**
 homocitrullinuria 231–2
 homocystinuria 144–50
 clinical abnormalities **145**, 145–8, **146**, **147**
 genetics and pathogenesis 148–9, **149**

- homocystinuria – *cont.*
 methylmalonic aciduria and *see*
 methylmalonic aciduria and
 homocystinuria
 treatment 149–50
- homogentisic acid 105, **106**, 106–7, 109, **109**
 homogentisic acid oxidase 105, **106**, 109
 defective activity *see* alkaptonuria
- homovanillic acid (HVA) levels, hypoxanthine–
 guanine phosphoribosyl transferase
 deficiency 494
- homozygous familial
 hypercholesterolemia 631, 632
 clinical abnormalities 632–4, **633**, **634**
 genetics and pathogenesis 634–6
 treatment 636–7
- human growth hormone *see* growth hormone
 therapy
- Hunter disease 555, 556, 572–7
 clinical abnormalities 572–6, **573**, **574**,
575
 genetics and pathogenesis 572, 576–7
 treatment 577
- Hurler disease 555, 556, 557, 558–64, 668
 clinical abnormalities 558–62, **559**, **560**,
561, **562**, **563**
 genetics and pathogenesis 558, 563
 treatment 563–4
- Hurler–Scheie disease 556, 566–70
 clinical abnormalities 567, 567–9, **568**, **569**
 genetics and pathogenesis 569–70
 treatment 570
- hydrocephalus
 Hunter disease 575
 Hurler–Scheie disease 568
 α -mannosidosis 746
 Maroteaux–Lamy disease 600
 Sly disease 606
- hydrogen sulfide 825
- hydrops fetalis 840
 galactosialidosis 753, 754
 Gaucher disease 702
- 3-hydroxy-3-methylglutaryl (HMG) CoA
 lyase 325, 326, **326**, 329
- 3-hydroxy-3-methylglutaryl (HMG) CoA lyase
 deficiency 247, **249**, 250, 325–31
 clinical abnormalities 326, 326–9, **327**, **328**,
329, **330**
 genetics and pathogenesis 325, 329–31
 treatment 331
- 3-hydroxy-3-methyl-5-hydroxypentanoic acid
 (mevalonic acid) 642, **642**, **643**, 645–6
- 3-hydroxy-3-methylglutaric (HMG)
 aciduria 325, 338
- 3-hydroxyacyl CoA dehydrogenase
 (HADH) 309, **309**, 310
- 3-hydroxyacyl CoA dehydrogenase (HADH)
 deficiency 309, 309–10
- 3-hydroxybutyrate
 accumulation 9
 excretion 6
- 3-hydroxybutyric acid levels
 carnitine transporter deficiency 254, 257,
257
 glycogenosis type III 450
 isovaleric acidemia 60
 medium-chain acyl CoA dehydrogenase
 deficiency 284
- 3-methylcrotonyl CoA carboxylase
 deficiency 76
- mitochondrial acetoacetyl-CoA thiolase
 (3-oxothiolase) deficiency 98
- mitochondrial DNA depletion 405
- 4-hydroxybutyric aciduria 5, 89–93
 clinical abnormalities 89, **90**, **91**, 91–2
 genetics and pathogenesis 90, 92–3
 treatment 93
- hydroxycobalamin, cobalamin C disease
 treatment 35, 37
- 3-hydroxydicarboxylic aciduria, long-chain
 L-3-hydroxyacyl CoA dehydrogenase
 deficiency 296, 297, 299
- 3-hydroxyglutaric acid 64, 70, 71
- D-2-hydroxyglutaric acid 79, **80**, 81–2
- L-2-hydroxyglutaric acid 81–2, 85, **85**, 87–8
- 3-hydroxyglutaric aciduria 840
- D-2-hydroxyglutaric aciduria 79–83
 clinical abnormalities 79–81, **81**
 genetics and pathogenesis 81–3
 treatment 83
- L-2-hydroxyglutaric aciduria 85–8
 clinical abnormalities 86, 86–7
 genetics and pathogenesis 87–8
- D-2-hydroxyglutaric dehydrogenase 79, **80**
- 5-hydroxyhexanoate 7
- 4-hydroxyisobutyric aciduria 83
- 3-hydroxyisovaleric acid 6, 6, 7
 biotinidase deficiency 52, 53
 holocarboxylase synthetase deficiency 40,
 42, 43, 44
- isovaleric acidemia 57, 59, 60
- 3-methylcrotonyl CoA carboxylase
 deficiency 76, 76
- p-hydroxyphenylacetic acid 168, **168**, 171, 175
- p-hydroxyphenyllactic acid 168, **168**, 171, 175
- p-hydroxyphenylpyruvic acid 168, **168**, 171,
 175
- 3-hydroxypropionic acid 42, 52
- hyperactivity
 adrenoleukodystrophy 460
 oculocutaneous tyrosinemia 167
 phenylketonuria 115, 119
- hyperacusis
 GM, activator deficiency 694
 Sandhoff disease 687
 Tay–Sachs disease 679
- hyperammonemia 191–6, 840
 argininemia 223, 224–5
 argininosuccinic aciduria 216, 217, 218,
 219, 220
 biotinidase deficiency 52
 carnitine palmitoyl transferase II deficiency,
 lethal neonatal 273
 carnitine transporter deficiency 254
 carnitine–acylcarnitine translocase
 deficiency 262, 264–5
 citrullinemia 210, **210**, 212, 213–14
 differential diagnosis 192, 192–3
 glutamine synthetase deficiency 242
 glutaric aciduria type I 66
 HHH syndrome 229, 230, 231, 232
 holocarboxylase synthetase deficiency 42,
 44
- 3-hydroxy-3-methylglutaryl CoA lyase
 deficiency 326, 329, 331
- isovaleric acidemia 58, 61
- long-chain L-3-hydroxyacyl CoA
 dehydrogenase deficiency 297
- lysine protein intolerance 236, 237,
 238
- medium-chain acyl CoA dehydrogenase
 deficiency 283, 285
- 3-methylcrotonyl CoA carboxylase
 deficiency 75
- methylmalonic acidemia 20, 21, 23, 28
- multiple acyl CoA dehydrogenase
 deficiency 317, 319
- propionic acidemia 9, 10, **11**, 14, 15
- pyruvate carboxylase deficiency 348, 351
 treatment 193–6, **194**, **194**
- hyperammonemic coma 192, **192**, 195–6
- argininemia 223
- argininosuccinic aciduria 216, 217, 218
- carbamyl phosphate synthetase
 deficiency 205–6
- citrullinemia 210, 211, 212
- HHH syndrome 230, 231
- ornithine transcarbamylase deficiency 197,
 198, 199
- hyperbilirubinemia, α 1-antitrypsin (AT)
 deficiency 804
- hypercalcemia 840
- hypercalciuria, von Gierke disease 432
- hyperchloremic acidosis 835
- hypercholesterolemia
 familial *see* familial hypercholesterolemia
 von Gierke disease 431
- hyperchylomicronemia
 apolipoprotein C-II deficiency 653
 lipoprotein lipase deficiency 648, 649, 650,
 650, 652
- hyperglycemia
 isovaleric acidemia 58–9
 mitochondrial acetoacetyl-CoA thiolase
 deficiency 96, 98
- hyperglycinemia 8
 nonketotic *see* nonketotic hyperglycinemia
- propionic acidemia 14
- hyperglycinuria, mitochondrial acetoacetyl-
 CoA thiolase deficiency 97
- hypergonadotropic hypogonadism,
 galactosemia 418
- hyperhidrosis, oculocutaneous
 tyrosinemia 166
- hyperimmunoglobulin D and periodic fever
 syndrome (HIDS) 643, 645, 646
- hyperimmunoglobulin D, mevalonic
 aciduria 645
- hyperinsulinemic
 hypoglycemia, 3-hydroxyacyl CoA
 dehydrogenase deficiency 310
- hyperkeratosis, oculocutaneous
 tyrosinemia 166, **166**
- hyperkinetic behavior, 4-hydroxybutyric
 aciduria 92
- hyperlipidemia 650
 apolipoprotein C-II deficiency 653
 carnitine palmitoyl transferase I
 deficiency 269, 271
 familial hypercholesterolemia *see* familial
 hypercholesterolemia
- lipoprotein lipase deficiency 649, 650, 651,
 652
- von Gierke disease 431

- hyperlipoproteinemia type IIb, cholesteryl ester storage disease 736
- hyperlordosis, mitochondrial DNA polymerase deficiency 407
- hyperlysinemia
propionic acidemia 14
pyruvate carboxylase deficiency 348
- hyperlysinuria, propionic acidemia 14
- hyperornithinemia 231
- hyperornithinemia, hyperammonemia,
homocitrullinuria syndrome *see* HHH
(hyperornithinemia, hyperammonemia,
homocitrullinuria) syndrome
- hyperphenylalaninemia 115, 116, 117, 118
- hyperphenylalaninemia and defective
metabolism of tetrahydrobiopterin 123–
31
clinical abnormalities 125–8, **126**, **127**
diagnosis 130
genetics and pathogenesis 128–9
treatment **130**, 130–1
- hyperpyrexia crises, glutaric aciduria type I 66
- hypersensitivity, Krabbe disease 727
- hypertension, argininosuccinic aciduria 218
- hypertension, elevated liver enzymes and low
platelets (HELLP) syndrome, long-chain
L-3-hydroxyacyl CoA dehydrogenase
deficiency 296, 297
- hypertonia
argininemia 223, 224
argininosuccinic aciduria 216
carbamyl phosphate synthetase
deficiency 206
CDG type If 792
citrullinemia 211
D-2-hydroxyglutaric aciduria 80
hyperphenylalaninemia and defective
metabolism of tetrahydrobiopterin 125
mitochondrial DNA depletion 406
phenylketonuria 115
propionic acidemia 10
- hypertriglyceridemia
apolipoprotein C-II deficiency 653
lipoprotein lipase deficiency 648, 650, 651,
652
- hypertyrosinemia 840
- hyperuricemia
Gaucher disease 699
hypoxanthine–guanine phosphoribosyl
transferase deficiency 488, 489, 493,
493
Lesch–Nyhan disease 483, 486–7
medium-chain acyl CoA dehydrogenase
deficiency 283
methylmalonic acidemia 23
phosphoribosylpyrophosphate synthetase
abnormalities 504, 505
von Gierke disease 431, 432
see also uric acid levels
- hyperventilation
fructose-1,6-diphosphatase deficiency 355
lactic acidemias 343
mitochondrial acetoacetyl-CoA thiolase
deficiency 96
- hypoalbuminemia, CDG type Ih 793
- hypocalcemia
CDG type Ig 793
isovaleric acidemia 58
- hypocitrullinemia 193
- hypodense myelin, propionic acidemia 10
- hypoglycemia
3-hydroxy-3-methylglutaryl CoA lyase
deficiency 326–7, 329, 330, 331
ethylmalonic encephalopathy 822
fatty acid oxidation disorders 247, **249**, 251
fructose-1,6-diphosphatase deficiency 354,
355, 356, 357
glutaric aciduria type I 66
glycogenosis type III 449–50, 452
hepatorenal tyrosinemia 172, 173, 174, 175
histidinuria 544
3-hydroxyacyl CoA dehydrogenase
deficiency 309, 310
lactic acidemias 343
maple syrup urine disease 155
3-methylcrotonyl CoA carboxylase
deficiency 75
methylmalonic acidemia 20, 21
mitochondrial DNA depletion 404, 405,
406, 409
multiple acyl CoA dehydrogenase
deficiency 316, 317, 319, 321
propionic acidemia 9
pyruvate carboxylase deficiency 348, 350,
351
von Gierke disease 429, 430, 431, 433, 434
Wolman disease 736
see also hypoketotic hypoglycemia
- hypogonadism
CDG type Ik 793
congenital disorders of glycosylation, type
Ia 784
cystinosis 534
galactosemia 418
Kearns–Sayre syndrome 394
- hypohidrosis, Fabry disease 660–1
- hypoketotic hypoglycemia 281, 840
carnitine palmitoyl transferase I
deficiency 268
carnitine palmitoyl transferase II deficiency,
lethal neonatal 273
carnitine transporter deficiency 254, 257,
257, 258
carnitine–acylcarnitine translocase
deficiency 261, 264
long-chain L-3-hydroxyacyl CoA
dehydrogenase deficiency 295, 296, 297
medium-chain acyl CoA dehydrogenase
deficiency 282, 283, 284, 286
multiple acyl CoA dehydrogenase
deficiency 319
short-chain 3-hydroxyacyl CoA
dehydrogenase deficiency 309
very long-chain acyl CoA dehydrogenase
deficiency 290, 292
- hypokinesia, tyrosine hydroxylase
deficiency 139, 140
- hypomagnesemia, Kearns–Sayre syndrome
394
- hypomethioninemia 36
- hypoparathyroidism, Kearns–Sayre
syndrome 394
- hypophosphatemia 841
cystinosis 532, 533
hepatorenal tyrosinemia 174
- hypothermia 3
- 3-hydroxy-3-methylglutaryl CoA lyase
deficiency 326
- argininosuccinic aciduria 216
- biotinidase deficiency 50
- carbamyl phosphate synthetase
deficiency 206
- isovaleric acidemia 58
- hypothyroidism, cystinosis 534
- hypotonia
arginine:glycine amidinotransferase
deficiency 828
argininosuccinic aciduria 216
aromatic L-amino acid decarboxylase
deficiency 136, 137
biotinidase deficiency 50, 51
Canavan disease 811, 812
carnitine transporter deficiency 254
carnitine–acylcarnitine translocase
deficiency 261–2
CDG type Ic 789
CDG type Ie 791
CDG type If 791
CDG type Ig 793
CDG type IL 793
CDG type In 793
CDG type Ip 794
CDG type Iq 794
CDG type IIb 794
CDG type IIc 794
CDG type IId 794
CDG type IIe 794
CDG type IIg 795
CDG type IIh 795
CDG type III 795
cobalamin C disease 34
congenital disorders of glycosylation, type
Ia 782
creatine transporter deficiency 828
cytosolic acetoacetyl CoA thiolase
deficiency 100
ethylmalonic encephalopathy 820
glutaric aciduria type I 65
glycogenosis type III 450
GM₁ gangliosidosis 667, 668
guanidinoacetate methyltransferase
deficiency 828
holocarboxylase synthetase deficiency 42
4-hydroxybutyric aciduria 89, 91, 92, 93
D-2-hydroxyglutaric aciduria 79, 80
hyperphenylalaninemia and defective
metabolism of tetrahydrobiopterin 125
isovaleric acidemia 59
Lesch–Nyhan disease 484
long-chain L-3-hydroxyacyl CoA
dehydrogenase deficiency 295, 296
metachromatic leukodystrophy 761
3-methylcrotonyl CoA carboxylase
deficiency 75
methylmalonic acidemia 22
mevalonic aciduria 644
mitochondrial acetoacetyl-CoA thiolase
deficiency 96
mitochondrial DNA depletion 405, 406, 407
NARP 389, 390
neonatal adrenoleukodystrophy **470**, 472
Niemann–Pick disease 710, 711
Niemann–Pick type C disease 720
nonketotic hyperglycinemia 181, **181**

- hypotonia – *cont.*
 propionic acidemia 9, 10, **10**
 Sandhoff disease 686
 short/branched chain acyl CoA
 dehydrogenase deficiency 312
 short-chain 3-hydroxyacyl CoA
 dehydrogenase deficiency 309
 short-chain acyl CoA dehydrogenase
 deficiency 304
 Tay–Sachs disease 679
 hypotonia–cystinuria syndrome 527, 528
 hypouricemia 841
 hypoxanthine–guanine phosphoribosyl
 transferase (HPRT) **483**, 490, **492**
 hypoxanthine–guanine phosphoribosyl
 transferase (HPRT) deficiency 483–94
 clinical abnormalities 483–90, **484**, **485**,
486, **487**, **489**, **490**
 genetics and pathogenesis 490–4, **492**, **493**
 treatment 494
 hypoxemia, α 1-antitrypsin (AT)
 deficiency 805
 hypsarrhythmia, D-2-hydroxyglutaric
 aciduria 80
- I-cell disease 613–18
 clinical abnormalities **613**, **614**, 614–17,
615, **616**, **617**
 genetics and pathogenesis **614**, 617–18
 treatment 618
 ichthyosis 841
 acute neuronopathic Gaucher disease 702
 holocarboxylase synthetase deficiency 41
 multiple sulfatase deficiency **771**, **772**
 iduronate sulfatase 572, **572**, 576
 iduronate sulfatase deficiency *see* Hunter
 disease
 α -L-iduronidase 558, **558**, 563, 566
 α -L-iduronidase deficiency
 Hurler disease *see* Hurler disease
 Hurler–Scheie disease *see* Hurler–Scheie
 disease
 Scheie disease *see* Scheie disease
 IgD levels, mevalonic aciduria 645
 Imerslund–Grasbeck B₁₂ intestinal absorptive
 defect 36, **37**
 immunodeficiency
 adenosine deaminase deficiency 507–8,
 509, 510
 biotinidase deficiency 51
 orotic aciduria 519
 immunologic abnormalities
 adenine phosphoribosyl-transferase (APRT)
 deficiency 499
 α 1-antitrypsin deficiency 806
 biotinidase deficiency 51
 holocarboxylase synthetase deficiency 42
 lysinuric protein intolerance 237
 α -mannosidosis 747
 indicanuria, Hartnup disease 541
 indolyluria 541–2, **542**
 infantile parkinsonism, tyrosine hydroxylase
 deficiency 139, 140
 infantile Refsum disease 469, 472, 475
 infections
 adenosine deaminase deficiency 507–8, 509
 I-cell disease 616
 α -mannosidosis 747
 methylmalonic acidemia 20
 orotic aciduria 519
 Pearson syndrome 399
 propionic acidemia 9
see also respiratory infections
 inflammatory bowel disease, glycogenosis type
 Ib 432
 inguinal hernias
 galactosialidosis 753
 Hurler disease 558, 560, 564
 Morquio syndrome 591
see also hernias
 insomnia
 aromatic L-amino acid decarboxylase
 deficiency 137
 glutaric aciduria type I 66
 Sanfilippo disease 583
 insulin therapy, maple syrup urine disease 158,
 159
 intelligence quotient (IQ)
 argininosuccinic aciduria 220
 congenital disorders of glycosylation, type
 Ia 783
 galactosemia 417, 418
 homocystinuria 147
 Lesch–Nyhan disease 485
 Menkes disease 550
 methylmalonic acidemia 22
 mevalonic aciduria 643–4
 mucopolipidosis III 625
 neonatal hyperammonemia 195
 phenylketonuria 113, 115, 118, 119, 120
 propionic acidemia 10
 intermediate branched-chain ketoaciduria 156
 intermediate hyperhomocysteinemia 155–6
 intestinal bacterial metabolites 6, 6–7
 intestinal bleeding *see* gastrointestinal bleeding
 intracranial hemorrhages, D-2-hydroxyglutaric
 aciduria 81
 inverted nipples 782, 841
 CDG Ia 782, **782**
 CDG type Ig 793
 CDG type Ip 794
 methylmalonic acidemia 21
 propionic acidemia 11, **13**
 iridodonesis, homocystinuria 145
 irritability
 acute neuronopathic Gaucher disease 702
 argininemia 223
 aromatic L-amino acid decarboxylase
 deficiency 137
 Canavan disease 811, 813
 carbamyl phosphate synthetase
 deficiency 206
 cobalamin C disease 34
 fructose-1,6-diphosphatase deficiency 355
 glutaric aciduria type I 65, 66
 HHH syndrome 229
 holocarboxylase synthetase deficiency 42
 D-2-hydroxyglutaric aciduria 80
 hyperphenylalaninemia and defective
 metabolism of tetrahydrobiopterin 126
 Krabbe disease 726
 ornithine transcarbamylase deficiency 199
 phenylketonuria 113
 isoleucine intolerance, methylmalonic
 acidemia 20
 isoleucine metabolism **152**
 isovaleric acidemia 57–61, 338
 clinical abnormalities **58**, 58–9, **59**, **60**
 genetics and pathogenesis 57, 59–61, **60**
 treatment 61
 isovaleryl CoA dehydrogenase **57**
 isovalerylglycine 57, **57**, 59, 60–1
- jaundice
 α 1-antitrypsin (AT) deficiency 804
 CDG type IIe 794
 cholestatic 837
 galactosemia 415, 418, 419
 hepatorenal tyrosinemia 172, 173
 long-chain L-3-hydroxyacyl CoA
 dehydrogenase deficiency 296
 Niemann–Pick type A disease 709
 Niemann–Pick type C disease 718, 720
see also cholestatic jaundice
 joint abnormalities
 GM₁ gangliosidosis 668
 I-cell disease 615
 Maroteaux–Lamy disease 598
 Morquio disease 589, **590**
 mucopolipidosis III 622, 623
 Sly disease 606
 joint pain, mevalonic aciduria 643, 645
 joint replacement
 alkaptanuria 109
 Gaucher disease 705
 joint stiffness
 GM₁ gangliosidosis 668
 Hunter disease 574, 575, 577
 Hurler–Scheie disease 568
 mucopolipidosis III 623
 Scheie disease 567
 juvenile galactosialidosis 753, 755, 756
 juvenile GM₂ gangliosidosis 679, 681
- Kearns–Sayre syndrome 393–6, 398
 clinical abnormalities **393**, 393–4, **394**
 genetics and pathogenesis **395**, 395–6, **396**
 treatment 396
 Kearns–Shy syndrome 377
 keratan sulfate
 GM₁ gangliosidosis 669
 Morquio disease 588, **588**, 593, 594
 keratan sulfatase, Morquio disease 588, 593
 keratitis, oculocutaneous tyrosinemia 165, 166
 keratoconjunctivitis, biotinidase deficiency 49
 keratosis palmaris et plantaris 164
 ketoacidosis
 biotinidase deficiency 52
 lactic acidemia 369
 3-methylcrotonyl CoA carboxylase
 deficiency 75, 76–7
 methylmalonic acidemia 20, 23, 27
 mitochondrial acetoacetyl-CoA thiolase
 deficiency 96, 97, 99, 100
 propionic acidemia 9, 10, 15
 pyruvate carboxylase deficiency 348, 350,
 351
 ketonuria
 carnitine transporter deficiency 254
 isovaleric acidemia 58
 von Gierke disease 431
 ketosis 6
 biotinidase deficiency 50
 glutaric aciduria type I 66, 70

- holocarboxylase synthetase deficiency 41, 44
 hyperammonemia 192
 metabolic acidosis and 842
 3-methylcrotonyl CoA carboxylase deficiency 75, 76–7
 methylmalonic acidemia 20
 mitochondrial acetoacetyl-CoA thiolase deficiency 95, 96, 98, 100
 propionic acidemia 9
 von Gierke disease 431
 ketostix testing
 methylmalonic acidemia 20
 propionic acidemia 15
 ketotic hyperglycinemia 8
 kidneys *see entries beginning renal*
 kinky hair, Menkes disease 546, 547
Klebsiella pneumoniae, dihydrofolate reductase deficiency 141
Klebsiella sepsis, propionic acidemia 9
 Krabbe disease 726–30
 clinical abnormalities 726–9, **727**, **728**
 genetics and pathogenesis 726, **726**, 729–30
 nonclassic/late-onset 728
 treatment 730
 Kussmaul breathing
 holocarboxylase synthetase deficiency 41
 methylmalonic acidemia 20
 kuan 118, 130
 kyphoscoliosis
 α -mannosidosis 746, **747**
 Maroteaux–Lamy disease 600
 mevalonic aciduria 644
 see also scoliosis
 kyphosis
 congenital disorders of glycosylation, type Ia 784
 GM₁ gangliosidosis, adult 671
 GM₁ gangliosidosis, infantile 668, **669**
 Hunter disease 574
 Maroteaux–Lamy disease 599
 Morquio disease **589**

L2HGDH gene 88
 lacrimation, oculocutaneous tyrosinemia 165
 lactescence, lipoprotein lipase deficiency **648**, 648–9
 D-lactic acid 6–7
 L-lactic acid 340, **340**, 342
 lactic acidemia(s) **4**, 6, 337–44, 841
 2-oxoglutarate dehydrogenase deficiency 369, 371, 372
 biotinidase deficiency 52, 53
 branched chain oxoacid dehydrogenase deficiency 371
 clinical abnormalities **343**, 343–4
 diagnostic work-up 338–43, **339**, **343**
 ethylmalonic encephalopathy 822
 fructose-1,6-diphosphatase deficiency *see* fructose-1,6-diphosphatase (FDP) deficiency
 holocarboxylase synthetase deficiency 42, 44
 Kearns–Sayre syndrome *see* Kearns–Sayre syndrome
 long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 297, 299
 MELAS *see* mitochondrial encephalomyelopathy, lactic acidosis and stroke-like episodes (MELAS)
 MERRF disease *see* myoclonic epilepsy and ragged red fiber (MERRF) disease
 mitochondrial DNA depletion 407, 409
 multiple acyl CoA dehydrogenase deficiency 319
 NARP *see* neurodegeneration, ataxia, and retinitis pigmentosa (NARP)
 pathogenesis 344
 Pearson syndrome *see* Pearson syndrome
 pyruvate carboxylase deficiency *see* pyruvate carboxylase deficiency
 pyruvate dehydrogenase complex deficiency *see* pyruvate dehydrogenase complex deficiency
 treatment 344
 von Gierke disease 431
 lactic acidosis
 3-hydroxy-3-methylglutaryl CoA lyase deficiency 329
 Kearns–Sayre syndrome 394
 MELAS 378
 pyruvate carboxylase deficiency 348, 350, 351
 lactic aciduria 6
 holocarboxylase synthetase deficiency 42
 lamellar ichthyosis, acute neuronopathic
 Gaucher disease 702
 late infantile galactosialidosis 753, 754, 756
 late infantile/juvenile GM₁ gangliosidosis 669–70, **670**, **671**
 LCHAD (long-chain L-3-hydroxyacyl CoA dehydrogenase) 250, 295, **295**, 298
 LCHAD deficiency *see* long-chain L-3-hydroxyacyl CoA dehydrogenase (LCHAD) deficiency
 learning disability
 L-2-hydroxyglutaric aciduria 86
 MELAS 376
 see also developmental delay; mental impairment
 Legg–Perthes disease 589
 Leigh syndrome 388, 390, 841
 lactic acidemia 342, 343, **343**, 344
 NARP 390, 391
 pyruvate carboxylase deficiency 348
 pyruvate dehydrogenase complex deficiency 361, 362, 363
 lens subluxation, homocystinuria 145, **145**, 150
 lenticular opacity 836
 multiple sulfatase deficiency 773
 see also cataracts
 Lesch–Nyhan disease 483–94
 clinical abnormalities 483–90, **484**, **485**, **486**, **487**, **489**, **490**
 genetics and pathogenesis 483, 490–4, **492**, **493**
 treatment 494
 lethargy
 argininosuccinic aciduria 216, 217
 carbamyl phosphate synthetase deficiency 205, 206
 citrullinemia 210, 212
 cobalamin C disease 34
 HHH syndrome 229, 230
 4-hydroxybutyric aciduria 92
 D-2-hydroxyglutaric aciduria 80
 medium-chain acyl CoA dehydrogenase deficiency 282, 283
 3-methylcrotonyl CoA carboxylase deficiency 75
 mitochondrial acetoacetyl-CoA thiolase deficiency 96, 98
 nonketotic hyperglycinemia 181
 ornithine transcarbamylase deficiency 192, 198, 199
 short/branched chain acyl CoA dehydrogenase deficiency 312
 very long-chain acyl CoA dehydrogenase deficiency 290
 leucine metabolism **152**
 leucine restriction, isovaleric acidemia management 61
 leukocyte adhesion defect (LAD) type II 794
 leukodystrophy
 adrenoleukodystrophy 462, **462**
 neonatal adrenoleukodystrophy 474
 leukopenia 841
 Gaucher disease 699
 isovaleric acidemia 59
 lysinuric protein intolerance 236
 leukotriene levels, mevalonic aciduria 646
 Lewis variant, Niemann–Pick disease 710, **711**
 linear growth, phenylketonuria 119
 lipemia retinalis 651, **651**
 lipid storage disorders 659–777
 cholesteryl ester storage disease *see* cholesteryl ester storage disease
 Fabry disease *see* Fabry disease
 fucosidosis 740–2, **741**, **742**
 galactosialidosis *see* galactosialidosis
 Gaucher disease *see* Gaucher disease
 GM₁ gangliosidosis *see* GM₁ gangliosidosis
 GM₂ activator deficiency *see* GM₂ activator deficiency
 Krabbe disease *see* Krabbe disease
 α -mannosidosis *see* α -mannosidosis
 β -mannosidosis 748–9
 metachromatic leukodystrophy *see* metachromatic leukodystrophy (MLD)
 multiple sulfatase deficiency *see* multiple sulfatase deficiency (MSD)
 Niemann–Pick disease *see* Niemann–Pick disease
 Niemann–Pick type C disease *see* Niemann–Pick type C disease
 Sandhoff disease *see* Sandhoff disease
 Tay–Sachs disease *see* Tay–Sachs disease
 Wolman disease *see* Wolman disease
 lipid storage myopathy, carnitine transporter deficiency 255
 lipoamide dehydrogenase **368**, 371
 deficiency 368, 371
 lipotrophy, congenital disorders of glycosylation, type Ia 782
 lipoic acid 368, **369**
 lipoic acid therapy 372
 lipomas, MERRF disease 383
 liponecrosis, lysinuric protein intolerance 237
 lipoprotein electrophoresis, lipoprotein lipase deficiency 652
 lipoprotein lipase deficiency 648–54
 clinical abnormalities **648**, 648–51, **649**, **651**
 genetics and pathogenesis 651–3
 treatment **653**, 653–4

- lipoprotein patterns, inherited
hyperlipidemias 649, 650
- lithotripsy, cystinuria 529
- liver
Wolman disease 736
see also entries beginning hepatic
- liver disease
 α 1-antitrypsin (AT) deficiency 803–6, **805**, **806**
see also specific liver diseases
- liver failure *see* hepatic failure
- liver transplantation
 α 1-antitrypsin (AT) deficiency 807
hepatorenal tyrosinemia 176
homozygous familial
hypercholesterolemia 636
maple syrup urine disease 159–60
methylmalonic acidemia 28
Niemann–Pick type C disease 722
ornithine transcarbamylase deficiency 201–2
propionic acidemia 16
pyruvate carboxylase deficiency 348, 351, 352
von Gierke disease 434
- L-lactic acid 340, **340**, 342
- loading tests, lactic acidemias 340
- long-chain 3-oxoacyl CoA thiolase (LCKAT) 295
deficiency 295–6
- long-chain L-3-hydroxyacyl CoA dehydrogenase (LCHAD) 250, 295, **295**, 298
- long-chain L-3-hydroxyacyl CoA dehydrogenase (LCHAD) deficiency 6, 250, 251, 295–9
clinical abnormalities **296**, 296–7, **298**
genetics and pathogenesis **295**, 297–9
treatment 299
- Lorenzo's oil 465
- low density lipoprotein (LDL) 635
- low density lipoprotein (LDL)
apheresis, homozygous familial
hypercholesterolemia 636–7
- low density lipoprotein (LDL) cholesterol levels, familial hypercholesterolemia 634
- low density lipoprotein receptor (LDLR) **632**, 635
mutations 631, 632, 632, 634, 635–6
- lymphedema, Fabry disease 659, 660, 661
- lymphocytes, vacuolated *see* vacuolated lymphocytes
- lymphocytosis, cerebrospinal fluid 836
- lymphopenia, adenosine deaminase deficiency 508
- lysine **525**
carbamyl phosphate synthetase deficiency 207
HHH syndrome 230, 231, 232
multiple acyl CoA dehydrogenase deficiency 320
propionic acidemia 14
- lysine excretion, cystinuria 525, 527, 528
- lysine supplementation
argininemia 226
HHH syndrome 232
lysine protein intolerance 238
- lysine tRNA, mutations 382, **382**, 384
- lysine protein intolerance 191, 235–8
clinical abnormalities 235–7, **236**
genetics and pathogenesis **235**, 237–8
treatment 238
- lysosomal α -1,4-glucosidase 438, **438**, 442–3
deficiency *see* Pompe disease
- lysosomal acid lipase *see* acid lipase
- lysosomal β -galactosidase *see* β -galactosidase
- lysosomal hydrolase deficiency 618, 621, 625, 678
see also specific disorders; specific enzymes
- lysosomal inclusions
congenital disorders of glycosylation, type Ia 784
GM₁ gangliosidosis 669
Maroteaux–Lamy disease 601
- lysosomal protective protein/cathepsin A *see* PPCA (protective protein/cathepsin A)
- lysyl oxidase deficiency 550
- macrocephaly 841–2
Canavan disease 811, 812, **812**
glutaric aciduria type I 64
GM₁ gangliosidosis 668
4-hydroxybutyric aciduria 91
D-2-hydroxyglutaric aciduria 79–80
L-2-hydroxyglutaric aciduria 87
Krabbe disease 728
 α -mannosidosis 745
multiple sulfatase deficiency **772**, 773
neonatal adrenoleukodystrophy 470
pyruvate carboxylase deficiency 350
Sly disease 606
- macroglossia
Hurler–Scheie disease **569**
Pompe disease 441, **441**
- macular degeneration, Tay–Sachs disease 679, 680
- magnetic resonance imaging (MRI)
adrenoleukodystrophy **461**, 461–2, **462**
biotinidase deficiency 52
Canavan disease 813
CDG type Ie 791, 792
cobalamin C disease 35, **35**
creatine transporter deficiency 829, **829**
ethylmalonic encephalopathy 822, **824**
galactosemia 418, 419
glutamine synthetase deficiency 242, **243**
glutaric aciduria type I 64, 67, **68**
guanidinoacetate methyltransferase deficiency 828
holocarboxylase synthetase deficiency 42
3-hydroxy-3-methylglutaryl CoA lyase deficiency 326, **328**
D-2-hydroxyglutaric aciduria 80
L-2-hydroxyglutaric aciduria 87
isovaleric acidemia 59, **60**
maple syrup urine disease 155
MELAS 375, **376**
metachromatic leukodystrophy 761, **762**, 763
methylmalonic acidemia 22–3, **23**
mitochondrial acetoacetyl-CoA thiolase deficiency 97, **97**
multiple acyl CoA dehydrogenase deficiency 319, **319**
NARP 390
nonketotic hyperglycinemia 182, **182**
- Pearson syndrome 401
- propionic acidemia 10, **11**
- pyruvate carboxylase deficiency 350
- 6-pyruvoyltetrahydropterin synthase deficiency 127, **127**
see also neuroimaging
- magnetic resonance spectroscopy (MRS)
arginine:glycine amidinotransferase (AGAT) deficiency 828
creatine transporter deficiency 828, **828**, 829
Fabry disease 661
guanidinoacetate methyltransferase deficiency 828
metachromatic leukodystrophy (MLD) 763
malar flush, homocystinuria **145**, 146
male fertility, phenylketonuria 120
maleylacetoacetic acid, hepatorenal tyrosinemia **172**, 175–6
malignant hyperthermia, carnitine palmitoyl transferase II deficiency, late onset 278
malocclusion, mucopolipidosis III 624, **625**
MAN2B1 gene 745, 748
D-mannose therapy, CDG type Ib 797
 α -mannosidase 745, **745**, 748
 α -mannosidosis 745–8
clinical abnormalities 745–8, **746**, **747**
genetics and pathogenesis 748, **748**, **749**
treatment 748
 β -mannosidosis 748–9
maple syrup urine disease (MSUD) 152–60
clinical abnormalities **153**, 153–6, **154**, **155**, **159**
genetics and pathogenesis **152**, 156–8, 157
treatment 156, 158, 158–60
Maroteaux–Lamy disease 556, 597–602
clinical abnormalities 597–601, **598**, **599**, **600**, **601**
genetics and pathogenesis **597**, 601–2
treatment 602
mass spectrometry (MS)
gas chromatography and *see* gas chromatography–mass spectrometry (GCMS)
tandem *see* tandem mass spectrometry (MS/MS)
- maternal phenylketonuria (MPKU) 120
- McArdle disease 426, 426
- medications, carbohydrate content 357
- medium-chain acyl CoA dehydrogenase (MCAD) 281, 286
- medium-chain acyl CoA dehydrogenase (MCAD) deficiency 193, 247, **249**, 250, **250**, 253, 281–7
clinical abnormalities **282**, 282–5, **283**, **284**
genetics and pathogenesis **285**, 285–6, **286**
treatment **283**, 286–7
- medium-chain dicarboxylic aciduria 284, 287
- medium-chain triglyceride (MCT)
supplementation
long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 299
very long-chain acyl CoA dehydrogenase deficiency 292
- megalencephaly
glutaric aciduria type I 64
see also macrocephaly
- megaloblastic anemia 842
cobalamin C disease 34

- dihydrofolate reductase deficiency 141
 orotic aciduria 519
 meganeurites, Tay–Sachs disease 681
 MELAS *see* mitochondrial encephalomyelopathy, lactic acidosis and stroke-like episodes (MELAS)
 Menkes disease 546–50
 clinical abnormalities **546, 547, 547, 547–9, 548**
 genetics and pathogenesis 549–50
 treatment 550
 mental impairment
 adenylosuccinate lyase deficiency 515
 arginine:glycine amidinotransferase deficiency 828
 argininosuccinic aciduria 216, 217, 218, 220
 aromatic L-amino acid decarboxylase deficiency 137
 carbamyl phosphate synthetase deficiency 206
 CDG type IIa 794
 CDG type IIc 794
 CDG type III 795
 citrullinemia 212, 214
 cobalamin C disease 34, 35
 congenital disorders of glycosylation, type Ia 783
 creatine transporter deficiency 828, 829
 galactosemia 417, 418, 419, 421, 422
 galactosialidosis 754, 755
 glycogenosis type III 449–50
 Hartnup disease 541
 HHH syndrome 229, 230, 231
 homocystinuria 146–7
 3-hydroxy-3-methylglutaryl CoA lyase deficiency 326
 4-hydroxybutyric aciduria 89, 91–2
 D-2-hydroxyglutaric aciduria 79
 L-2-hydroxyglutaric aciduria 86
 I-cell disease 615
 Lesch–Nyhan disease 485
 lysinuric protein intolerance 236
 α -mannosidosis 745, 746
 maple syrup urine disease 153, 156
 mitochondrial acetoacetyl-CoA thiolase deficiency 96
 Niemann–Pick disease 710
 nonketotic hyperglycinemia 182
 oculocutaneous tyrosinemia 166–7
 ornithine transcarbamylase deficiency 199
 phenylketonuria 113, 116
 propionic acidemia 9
 pyruvate carboxylase deficiency 349
 Sanfilippo disease 580
 short/branched chain acyl CoA dehydrogenase deficiency 312
 Sly disease 606
 tyrosine hydroxylase deficiency 140
 mesaconic acid 60
 metabolic acidosis
 3-hydroxy-3-methylglutaryl CoA lyase deficiency 326, 329
 ketosis and 842
 pyruvate carboxylase deficiency 348, 349, 350, 351, 352
 pyruvate dehydrogenase complex deficiency 361, 363
see also ketoacidosis; lactic acidosis
- metachromasia
 Hurler disease 562
 Sanfilippo disease 584
 metachromatic leukodystrophy (MLD) 760–4
 clinical abnormalities **761, 761–3, 762**
 genetics and pathogenesis 760, **760, 763–4**
 treatment 764
see also multiple sulfatase deficiency (MSD)
- methionine
 hepatorenal tyrosinemia 175
 homocystinuria 144, 149
 methionine intolerance, methylmalonic acidemia 20
 methionine synthase **4, 20**
 methionine synthase deficiency 33, **33, 36**
 methotrexate 141–2
 2-methyl-3-hydroxybutyric acid 95, **95, 98, 100**
 2-methyl-3-oxovaleric acid 14
 2-methylacetoacetic acid 95, **95, 98**
 2-methylbutyryl CoA dehydrogenase deficiency 312–14, **313**
 2-methylbutyrylglutininuria, short/branched chain acyl CoA dehydrogenase deficiency 312, 314
 methylcitric acid 43, 52
 3-methylcrotonic acid, 3-hydroxy-3-methylglutaryl CoA lyase deficiency 331
 3-methylcrotonyl CoA carboxylase deficiency 74–7
 clinical abnormalities **75, 75**
 genetics and pathogenesis **74, 76, 76–7**
 treatment 77
 3-methylcrotonyl glycinuria *see* 3-methylcrotonyl CoA carboxylase deficiency
 3-methylcrotonylglycine biotinidase deficiency 52
 holocarboxylase synthetase deficiency 40, 42
 3-methylcrotonyl CoA carboxylase deficiency 74, 76, 76
 methylene tetrahydrofolate reductase deficiency 34, **548**
 3-methylglutaconic acid, carbamyl phosphate synthetase deficiency 207
 methylmalonic acid **4, 6, 24**
 excretion 19, 25
 methylmalonic acidemia diagnosis 24–6, **25, 25**
 methylmalonic acidemia 8, 19–28, 338
 clinical abnormalities 20–4, **21, 22, 23**
 differential diagnosis 19, 19–20
 genetics and pathogenesis **20, 24–7, 25, 25**
 treatment 27–8, 195
 methylmalonic aciduria 842
 mitochondrial DNA depletion 408
 methylmalonic aciduria and homocystinuria **33, 33–8**
 clinical abnormalities **34, 34–6, 37**
 differential diagnosis 34
 genetics and pathogenesis 36, 36–7, **37**
 treatment 37–8
 methylmalonyl CoA mutase **4**
 methylmalonyl CoA mutase deficiency
 methylmalonic acidemia 19, 20, **20, 24, 26**
 methylmalonic aciduria and homocystinuria **33, 33, 36**
 methylsuccinic acid 60, 304, 823, 824, 825
- 5-methyltetrahydrofolate (5-MTHF) 141
 metronidazole therapy
 ethylmalonic encephalopathy **823, 824, 825**
 lactic acidemias 340
 methylmalonic acidemia 27
 organic acidemias 7
 propionic acidemia 15–16
 mevalonate kinase **642, 643**
 defective activity *see* mevalonic aciduria
 mevalonic acid 642, **642, 643, 645–6**
 mevalonic aciduria 642–6
 clinical abnormalities **643, 643–5, 644, 646**
 genetics and pathogenesis **642, 643, 645–6**
 treatment 646
 microcephaly
 arginine:glycine amidinotransferase deficiency 828
 argininemia 223, **224**
 CDG type Id 790
 CDG type Ij 793
 CDG type Ik 793
 CDG type IL 793
 CDG type Ip 794
 CDG type Ig 793
 CDG type IIc 794
 CDG type IIg 795
 CDG type IIj 795
 cobalamin C disease 34, 35
 dihydrofolate reductase deficiency 141
 3-hydroxy-3-methylglutaryl CoA lyase deficiency 326
 4-hydroxybutyric aciduria 91
 Krabbe disease 728
 phenylketonuria 115, 120
 micrognathism, Hurler–Scheie disease 567, **568**
 micromelia, glutamine synthetase deficiency 241
 migraine, MELAS 374, 375–6, 378
 mineral transport disorders, Menkes disease *see* Menkes disease
 mipomersen, heterozygous familial hypercholesterolemia management 637
 mitochondrial acetoacetyl-CoA thiolase 95, **95, 96, 99**
 mitochondrial acetoacetyl-CoA thiolase deficiency 95–100
 clinical abnormalities 96–8, **97, 98**
 differential diagnosis 100, **100**
 genetics and pathogenesis **95, 98–9, 99**
 treatment 100
 mitochondrial disorders 337, 338, **339, 340, 342–3**
see also lactic acidemia(s); *specific disorders*
 mitochondrial DNA depletion 344, 404
 mitochondrial DNA depletion syndromes 404–10
 clinical abnormalities **405, 406, 406–8, 407**
 genetics and pathogenesis **408, 408–9**
 treatment 409–10
 mitochondrial DNA polymerase deficiency 404, 405, 407, 408–9
 mitochondrial encephalomyelopathy, lactic acidosis and stroke-like episodes (MELAS) 374–9, 383
 clinical abnormalities 374–8, **375, 376, 377, 378**

- mitochondrial encephalomyelopathy, lactic acidosis and stroke-like episodes (MELAS) – *cont.*
 genetics and pathogenesis 374, 375, 377, 378, 378–9
 treatment 379
- mitochondrial genome 374, 384, 398, 409
- MMACHC* gene 33
- Mongolian spot 842
 late infantile/juvenile GM₁ gangliosidosis 669, 670
- moniliasis
 methylmalonic acidemia 21, 21, 24
 propionic acidemia 11
- Moro reflex, maple syrup urine disease 153
- Morquio B disease
 clinical abnormalities 666, 671, 671, 673, 673, 674
 genetics and pathogenesis 672, 673, 673, 674
 treatment 674
- Morquio syndrome 500, 556, 588–94
 clinical abnormalities 589, 589–93, 590, 591, 592, 593
 genetics and pathogenesis 588, 593–4
 treatment 594
 type B *see* Morquio B disease
- motor weakness
 Sandhoff disease 686
 Tay–Sachs disease 679
see also muscle weakness
- mucocutaneous candidiasis, biotinidase deficiency 50
- mucopolipidoses 613–26
 pseudo-Hurler polydystrophy *see* mucopolipidosis III
 type II *see* I-cell disease
 type III *see* mucopolipidosis III
- mucopolipidosis III 621–6
 clinical abnormalities 621, 621–5, 622, 623, 624, 625
 genetics and pathogenesis 625–6
 treatment 626
- mucopolysaccharides, GM₁ gangliosidosis 668
- mucopolysaccharidoses 555–7, 556
 Hunter disease *see* Hunter disease
 Hurler disease *see* Hurler disease
 Hurler–Scheie disease *see* Hurler–Scheie disease
 Maroteaux–Lamy disease *see* Maroteaux–Lamy disease
 Morquio syndrome *see* Morquio syndrome
 Sanfilippo disease *see* Sanfilippo disease
 Scheie disease *see* Scheie disease
 Sly disease *see* Sly disease
- mucopolysaccharidosis
 multiple sulfatase deficiency 769, 770, 772
 type IH *see* Hurler disease
 type IHS *see* Hurler–Scheie disease
 type II *see* Hunter disease
 type III *see* Sanfilippo disease
 type IS *see* Scheie disease
 type IV *see* Morquio syndrome
 type IV B *see* Morquio B disease
 type VI *see* Maroteaux–Lamy disease
 type VII *see* Sly disease
- mucopolysacchariduria, multiple sulfatase deficiency 774
- multiple acyl CoA dehydrogenase deficiency (MADD) 247, 249, 251, 316–22
 clinical abnormalities 317–20, 318, 319, 320
 genetics and pathogenesis 316, 320–1
 treatment 321–2
- multiple carboxylase deficiency 5, 40, 41, 338
 biotinidase deficiency *see* biotinidase deficiency
 holocarboxylase synthetase deficiency *see* holocarboxylase synthetase deficiency
- multiple sulfatase deficiency (MSD) 769–77
 clinical abnormalities 770, 770–5, 771, 772, 773, 774, 775
 genetics and pathogenesis 769, 769, 775–6
 treatment 776–7
- muscle atrophy
 congenital disorders of glycosylation, type Ia 784
 glycogenosis type III 450
- muscle biopsy
 ethylmalonic encephalopathy 823
 lactic acidemias 339, 340, 341
- muscle weakness
 carnitine transporter deficiency 254, 255, 258
 carnitine–acylcarnitine translocase deficiency 261–2
 glycogenosis III 450
 Krabbe disease 728
 lactic acidemias 344
 NARP 388, 389, 390
 Pompe disease 441
- muscular pain, carnitine palmitoyl transferase II deficiency, late onset 277
- mutilation *see* self-injurious behavior
- myelin, hypodense, propionic acidemia 10
- myelination
 adrenoleukodystrophy 462, 464
 glutamine synthetase deficiency 242, 243
 maple syrup urine disease 155
 multiple acyl CoA dehydrogenase deficiency 319
 nonketotic hyperglycinemia 182
see also demyelination
- myelopathy
 cobalamin C disease 34
 Hurler–Scheie disease 568
 Maroteaux–Lamy disease 600
 Scheie disease 567
- myocardial infarction 842
- Fabry disease 661
 familial hypercholesterolemia 632
- myoclonic epilepsy and ragged red fiber (MERRF) disease 382–5
 clinical abnormalities 383, 383–4
 genetics and pathogenesis 382, 384–5
 treatment 385
- myoclonic seizures
 biotinidase deficiency 48
 hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin 126
 MERRF disease 383
see also seizures
- myoclonic spasms, HHH syndrome 229, 230
- myoglobinuria
 carnitine palmitoyl transferase II deficiency, late onset 277, 278
- long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 296
- short-chain 3-hydroxyacyl CoA dehydrogenase deficiency 309
- very long-chain acyl CoA dehydrogenase deficiency 290
- myopathic carnitine palmitoyl transferase deficiency 247
- myopathy
 carnitine palmitoyl transferase II deficiency, lethal neonatal 274
 cystinosis 534
 fatty acid oxidation disorders 247
 glycogenosis type II 438–9, 441–2, 444
 glycogenosis type III 450, 452, 453
 Kearns–Sayre syndrome 394
 medium-chain acyl CoA dehydrogenase deficiency 281, 283
 MELAS 375, 376, 378, 379
 metachromatic leukodystrophy 761
 3-methylcrotonyl CoA carboxylase deficiency 75
 mevalonic aciduria 644
- myopia
 biotinidase deficiency 51
 homocystinuria 145
 L-2-hydroxyglutaric aciduria 87
- nails, dystrophic, lysinuric protein intolerance 236
- NARP *see* neurodegeneration, ataxia, and retinitis pigmentosa (NARP)
- nausea, ethylmalonic–adipic aciduria 319
- necrolytic erythema, glutamine synthetase deficiency 242, 242
- neomycin therapy
 Hartnup disease 542
 lactic acidemias 340
 methylmalonic acidemia 27
 organic acidemias 7
 propionic acidemia 15–16
- neonatal adrenoleukodystrophy 459, 469–76
 clinical abnormalities 470, 470–4, 471, 472, 473
 genetics and pathogenesis 474–6
 treatment 476
- neonatal cholestasis, α 1-antitrypsin (AT) deficiency 804
- neonatal hepatitis, α 1-antitrypsin (AT) deficiency 804
- neonatal hyperammonemia, propionic acidemia 15
- neopterin 125, 128, 128, 129, 130
- nephrocalcinosis, von Gierke disease 432
- nephropathic cystinosis 533–4, 535–6
- neuraminidase deficiency,
 galactosialidosis 752, 753, 756
- neurodegeneration
 cobalamin C disease 35
 ethylmalonic encephalopathy 820
 Gaucher disease 698, 702
 glutaric aciduria type I 64
 GM₁ gangliosidosis 668
 GM₂ activator deficiency 694
 D-2-hydroxyglutaric aciduria 87
 Krabbe disease 726
 β -mannosidosis 748
 MERRF disease 384

- metachromatic leukodystrophy 761–3
 methylmalonic aciduria and
 homocystinuria 35
 mitochondrial DNA depletion 407
 multiple sulfatase deficiency 770
 Niemann–Pick type C disease 720, 722
 nonketotic hyperglycinemia 182
 Sandhoff disease 688
 Sanfilippo disease 580–1
see also specific manifestations
 neurodegeneration, ataxia, and retinitis
 pigmentosa (NARP) 388–91
 clinical abnormalities 388–91, **389, 390, 391**
 genetics and pathogenesis 388, 391
 treatment 391
 neuroimaging
 adrenoleukodystrophy **461, 461–2, 462**
 argininemia 223
 argininosuccinic aciduria 217
 Canavan disease 813, **814**
 citrullinemia 212
 ethylmalonic encephalopathy 822, **823, 824**
 GM₁ gangliosidosis 668
 Hunter disease 575
 Kearns–Sayre syndrome 394
 Krabbe disease 728
 α -mannosidosis 746
 MELAS 375, **376**
 Menkes disease 548
 metachromatic leukodystrophy 761, **762, 763**
 mevalonic aciduria 644
 multiple sulfatase deficiency 773–4
 NARP 390, **391**
 neonatal adrenoleukodystrophy 470
 pyruvate dehydrogenase complex
 deficiency 361
 see also computed tomography (CT);
 magnetic resonance imaging (MRI)
 neurologic crisis, hepatorenal tyrosinemia 174
 neuronal lipidosis
 GM₁ gangliosidosis 668
 GM₂ gangliosidosis 678
 neuronopathic Gaucher disease (type II) 698,
 698, 702
 neuropathy, peripheral *see* peripheral
 neuropathy
 neurotransmitters
 abnormalities *see* biogenic amine
 abnormalities
 analysis 5
 neurotrophin therapy, Fabry disease 662
 neutral lipid metabolism, lipoprotein lipase
 deficiency *see* lipoprotein lipase deficiency
 neutropenia 842
 glycogenosis type Ib 432
 methylmalonic acidemia 24
 Pearson syndrome 398, 399
 propionic acidemia 11
 neutrophilia, CDG type IIc 794
 nicotinamide supplementation, Hartnup
 disease 542
 nicotinic acid supplementation, Hartnup
 disease 542
 Niemann–Pick disease 708–15
 708–709 **709**
 clinical abnormalities **709, 709–12, 710, 711, 712, 713, 714**
 genetics and pathogenesis 708–9, **709, 712–15**
 Lewis variant 710, **711**
 Saudi variant 711
 treatment 715
 type A *see* Niemann–Pick type A disease
 type B *see* Niemann–Pick type B disease
 type C *see* Niemann–Pick type C disease
 type D 718, 721
 Niemann–Pick type A disease 708
 clinical abnormalities **709, 709–10, 710, 712, 713, 714**
 genetics and pathogenesis 709, 712–14
 treatment 715
 Niemann–Pick type B disease 708–9, 710–11,
 711, 712
 clinical abnormalities **709, 710, 710–11, 711, 712, 713, 714**
 genetics and pathogenesis 712–15
 Lewis variant 710, **711**
 Saudi variant 711
 treatment 715
 Niemann–Pick type C disease 708, 710,
 718–22
 clinical abnormalities 718–21, **719, 720**
 genetics and pathogenesis 718, 721–2
 treatment 722
 night blindness, NARP 388
 nitisinone therapy *see* NTBC (nitisinone)
 therapy
 N-linked glycosylation 787
 congenital disorders *see* congenital disorders
 of glycosylation (CDG)
 nocturnal nasogastric feeding, von Gierke
 disease 434
 nonimmune hydrops fetalis, Sly disease 607
 nonketotic hyperglycinemia 8, 180–5
 clinical abnormalities **181, 181–3, 182**
 genetics and pathogenesis 183–5, **184, 184, 185**
 treatment 185
 Norrbottnian form of Gaucher disease (type
 III) 698, 698, 702
 NPC1 gene 718, 721, 722
 NPC2 gene 718, 721
 NTBC (nitisinone) therapy **176**
 alkaptonuria 105, 109
 hepatorenal tyrosinemia 171, 176
 nuclear magnetic resonance (NMR), organic
 acid detection 5
 nystagmus
 Canavan disease 813
 CDG type If 791
 CDG type Ii 793
 cobalamin C disease 35
 obstructive airway disease
 α 1-antitrypsin (AT) deficiency 805
 Hunter disease 575, 577
 occipital horn disease 549
 ochronosis, alkaptonuria 107, **107, 108, 108**
 ochronotic arthritis, alkaptonuria 107, 108,
108
 ocular lesions
 Fabry disease **660, 661**
 oculocutaneous tyrosinemia **165, 165–6**
 see also specific ocular lesions
 oculocutaneous tyrosinemia 164–9
 clinical abnormalities 165–7, **166**
 genetics and pathogenesis **164, 167, 167–8, 168**
 treatment **168, 168–9**
 oculogyric crises
 aromatic L-amino acid decarboxylase
 deficiency 136
 tyrosine hydroxylase deficiency 140
 oculomotor apraxia, acute neuronopathic
 Gaucher disease 702
 odontoid abnormalities, multiple sulfatase
 deficiency 773
 odontoid hypoplasia
 Maroteaux–Lamy disease 601
 Morquio disease 591, **592, 593**
 multiple sulfatase deficiency 773, **773**
 Sly disease 606, 607
 odor 842–3
 hepatorenal tyrosinemia 172–3
 isovaleric acidemia 58
 maple syrup urine disease 152, 154–5
 multiple acyl CoA dehydrogenase
 deficiency 317
 phenylketonuria 113–14
 O-linked glycosylation 788
 olivopontocerebellar atrophy,
 adrenoleukodystrophy 461
 ophthalmoplegia
 Kearns–Sayre syndrome 393, 394
 lactic acidemias 344
 MELAS 377
 NARP 390
 Niemann–Pick type C disease 720
 opisthotonos
 argininemia 223, **224**
 carbamyl phosphate synthetase
 deficiency 206
 glutaric aciduria type I 64, 65
 holocarboxylase synthetase deficiency 42
 maple syrup urine disease 153, **153**
 nonketotic hyperglycinemia 181
 ornithine transcarbamylase deficiency 199
 optic atrophy 843
 adrenoleukodystrophy 460
 biotinidase deficiency 51, 52
 Canavan disease 813
 GM₁ gangliosidosis 668
 homocystinuria 145
 MERRF disease 383
 metachromatic leukodystrophy 761, 762
 methylmalonic acidemia 23
 Sandhoff disease 687, 688
 orange crystals, Lesch–Nyhan disease 483,
 488
 organic acid analysis 3, 5, 5–7, 6
 organic acidemias 3, 3–7
 classic presentation 3
 glutaric aciduria *see* glutaric aciduria, type I
 D-2-hydroxyglutaric aciduria *see* D-2-
 hydroxyglutaric aciduria
 isovaleric acidemia *see* isovaleric acidemia
 metabolic interrelations **4**
 3-methylcrotonyl CoA carboxylase
 deficiency *see* 3-methylcrotonyl CoA
 carboxylase deficiency
 3-methylcrotonyl glycinuria *see*
 3-methylcrotonyl CoA carboxylase
 deficiency

- organic acidemias – *cont.*
 methylmalonic acidemia *see* methylmalonic acidemia
 methylmalonic aciduria and homocystinuria *see* methylmalonic aciduria and homocystinuria
 mitochondrial acetoacetyl-CoA thiolase deficiency *see* mitochondrial acetoacetyl-CoA thiolase deficiency
 multiple carboxylase deficiency 40, 41
 biotinidase deficiency *see* biotinidase deficiency
 holocarboxylase synthetase deficiency *see* holocarboxylase synthetase (HCS) deficiency
 propionic acidemia *see* propionic acidemia
 ornithine 525
 ornithine excretion, cystinuria 525, 527
 ornithine levels
 argininemia 225
 multiple acyl CoA dehydrogenase deficiency 320
 ornithine supplementation
 argininemia 226
 HHH syndrome 232–3
 ornithine transcarbamylase (OTC) 197, 199, 200
 ornithine transcarbamylase (OTC)
 deficiency 191–2, 193, 197–202
 clinical abnormalities 195–6, 197–9, **198**
 genetics and pathogenesis 199–201, **201**
 treatment 195, 201, 201–2
 ornithinuria, HHS syndrome 231
 orotic aciduria 5, 193, 518–20, 843
 argininemia 226, **226**, 227
 citrullinemia 212
 clinical features 518–19, **519**, 519
 genetics and pathogenesis 518, 519–20
 HHH syndrome 231, 231
 ornithine transcarbamylase deficiency 199, 200
 treatment 520
 orotidyl decarboxylase 518, **518**
 deficiency 520
see also orotic aciduria
 orotidyl pyrophosphorylase 518, **518**
 deficiency 520
see also orotic aciduria
 osteoarthritis, alkaptonuria 105, 107–8, **108**
 osteopenia, homocystinuria **147**
 osteoporosis 843
 Gaucher disease 700, **701**
 homocystinuria 146
 lysinuric protein intolerance 236, 238
 methylmalonic acidemia 24
 Morquio syndrome 593
 Niemann–Pick disease 710
 propionic acidemia 11
 von Gierke disease 431
 otitis media, adenosine deaminase deficiency 508
 ovarian failure, galactosemia 418
 oxidative phosphorylation 341
 disorders 342, 382, 384, 385
 2-oxoglutarate dehydrogenase deficiency 369, 371, 372
 2-oxoglutaric acid excretion, D-2-hydroxyglutaric aciduria 82
 3-oxothiolase 95, **95**, **96**, 99
 deficiency *see* mitochondrial acetoacetyl-CoA thiolase deficiency
 pachymeningitis cervicalis 567
 pain
 carnitine palmitoyl transferase II deficiency, late onset 277
 elevated erythrocyte sedimentation rate and 843
 Fabry disease 659, 659–60, 661, 662
 hepatorenal tyrosinemia 174
 lipoprotein lipase deficiency 649–50, 654
 metachromatic leukodystrophy 761
 mevalonic aciduria 643, 645
 mucopolipidosis III 623, 626
 pallor, 3-hydroxy-3-methylglutaryl CoA lyase deficiency 326
 pamidronate therapy, mucopolipidosis III 626
 pancreatic insufficiency, Pearson syndrome 399, 401, 402
 pancreatitis 843
 chronic 837
 homocystinuria 148
 3-hydroxy-3-methylglutaryl CoA lyase deficiency 326, 329
 isovaleric acidemia 59
 lipoprotein lipase deficiency 650, 654
 lysinuric protein intolerance 237
 maple syrup urine disease 155
 MELAS 377
 methylmalonic acidemia 24
 propionic acidemia 11
 von Gierke disease 432
 pancytopenia
 dihydrofolate reductase deficiency 141
 α -mannosidosis 747
 methylmalonic acidemia 24
 methylmalonic acidemia and homocystinuria 34, 35, 36
 Niemann–Pick disease 710
 Pearson syndrome 398–9, 401
 propionic acidemia 9
 papilledema
 Hunter disease 575
 Maroteaux–Lamy disease 600
 parakeratosis, oculocutaneous tyrosinemia 166
 paralysis of upward gaze 843
 Kearns–Sayre syndrome 393, **393**
 Niemann–Pick type C disease 719, **720**
 paraparesis/paraplegia *see* spastic paraparesis/paraplegia
 paresthesia, hepatorenal tyrosinemia 174
 parkinsonism
 Gaucher disease 701–2
 tyrosine hydroxylase deficiency 139, 140
 PCSK9 gene mutations 631, 634–5, 636
 Pearson syndrome 394, 398–402
 clinical abnormalities 398–401, **399**, **400**
 genetics and pathogenesis **398**, 401–2
 treatment 402
 peau d'orange, congenital disorders of glycosylation, type Ia 782
 pectus carinatum, Morquio disease 589, **589**
 pectus excavatum, homocystinuria 146, **146**
 pellagra-like dermatosis 541
 penicillamine therapy, cystinuria 529, **529**
 perineal dermatitis, biotinidase deficiency **48**, 50
 perioral stomatitis, biotinidase deficiency 50
 peripheral neuropathy
 carnitine transporter deficiency 255
 congenital disorders of glycosylation, type Ia 783
 hepatorenal tyrosinemia 174
 Krabbe disease 728
 long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 297, 299
 MELAS 377
 MERRF disease 383
 metachromatic leukodystrophy 761, 762–3
 NARP 389, 390
 peritoneal dialysis, hyperammonemia 194
 pernicious anemia, methylmalonic acidemia 19, 19
 peroxisomal assembly factor 1 (PAF1) 469
 peroxisomal biogenesis disorders 469–76
 clinical abnormalities **470**, 470–4, **471**, **472**, **473**
 genetics and pathogenesis 474–6
 treatment 476
 peroxisomal β -oxidation, fatty acids **469**, **473**
 peroxisomal disorders 459–76
 adrenoleukodystrophy *see* adrenoleukodystrophy
 biogenesis *see* peroxisomal biogenesis disorders
 petechiae
 carnitine palmitoyl transferase I deficiency 269
 ethylmalonic encephalopathy **820**, 821
 PEX genes 470, 474–5
 phenylacetate therapy **194**
 carbamyl phosphate synthetase deficiency 207
 hyperammonemia 194, 194–5
 ornithine transcarbamylase deficiency 201
 phenylalanine **112**, 116, **117**, 118
 phenylalanine hydroxylase 112, 113, 115, 116, **123**, 124
 defective activity *see* phenylketonuria (PKU)
 phenylalanine metabolism **172**
 phenylalanine restriction, phenylketonuria management 118–19
 phenylbutyrate therapy
 argininemia 227
 carbamyl phosphate synthetase deficiency 207
 carnitine transporter deficiency 258
 citrullinemia 214
 hyperammonemia 195
 ornithine transcarbamylase deficiency 201, **201**
 phenylketonuria (PKU) 112–20
 clinical abnormalities 113–15, **114**, **118**
 diagnosis 116–18, **117**
 genetics and pathogenesis **112**, 115–16
 maternal 120
 treatment **118**, 118–19
 phenylpropionylglycine 284
 phosphaturia, hepatorenal tyrosinemia 173
 phosphomannomutase-2 781, **781**, 785
 phosphomannomutase-2 deficiency *see* congenital disorders of glycosylation, type Ia

- phosphomannose isomerase **781**, 794
 phosphomannose isomerase deficiency 788–9, 790, **791**, 795, 799
 phosphoribosylpyrophosphate (PRPP) synthetase 503, **503**
 phosphoribosylpyrophosphate (PRPP) synthetase abnormalities 503–5, **504**
 phosphorylase kinase deficiency 426, 427
 photophobia 843
 cystinosis 534, 535
 oculocutaneous tyrosinemia 165
 photosensitivity, Hartnup disease 541
 pica, Hunter disease 575
 pigmentary retinopathy
 cobalamin C disease 34
 long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 297, 299
 see also retinitis pigmentosa
 pigmentation
 alkaptonuria 107, **107**
 homocystinuria 145–6
 skin *see* skin pigmentation
 pili torti, Menkes disease 547, **548**, 549
 pipecolic aciduria, neonatal
 adrenoleukodystrophy 469, 476
 placental enlargement, Niemann–Pick disease 709
 plasmapheresis, homozygous familial hypercholesterolemia 636
 platyspondyly
 galactosialidosis 755
 homocystinuria 146
 Morquio disease 591
 PMM2 gene 785
 pneumonia
 adenosine deaminase deficiency 508
 Krabbe disease 728
 Niemann–Pick disease 709, 710, 711
 PNPO (pyridox(am)ine 5'-phosphate oxidase) deficiency 138, 138
 POLG gene mutations 404, 408, 409
 polycystic kidneys 843
 carnitine palmitoyl transferase II deficiency, lethal neonatal 273
 multiple acyl CoA dehydrogenase deficiency 317, 319
 polycystic ovaries, glycogenosis III 450
 polydipsia, cystinosis 533
 polymicrogyria, neonatal
 adrenoleukodystrophy 474
 polyuria
 cystinosis 533
 Fabry disease 661
 Pompe disease 426, 426, 438–44
 clinical abnormalities **439**, 439–42, **440**, **441**, **442**
 genetics and pathogenesis **438**, 442–4
 treatment 444
 posturing
 argininosuccinic aciduria 216
 citrullinemia 211
 propionic acidemia 10
 see also decerebrate posturing
 Potter syndrome, multiple acyl CoA dehydrogenase deficiency 317
 PPCA (protective protein/cathepsin A) 752, 755, 756
 abnormality 752, 753, 755, 756
 pregnancy
 carnitine palmitoyl transferase I deficiency 269
 carnitine transporter deficiency 258
 cystinosis 534
 galactosemia 418
 holocarboxylase synthetase (HCS) deficiency 44
 homocystinuria 148
 isovaleric acidemia 59
 lipoprotein lipase deficiency 650
 Maroteaux–Lamy disease 600
 methylmalonic acidemia 24
 mitochondrial acetoacetyl-CoA thiolase deficiency 97
 oculocutaneous tyrosinemia 167, 169
 phenylketonuria 120
 6-pyruvoyltetrahydropterin synthase deficiency 131
 Tay–Sachs disease monitoring 683, 683
 premature ageing, congenital disorders of glycosylation, type Ia 784
 prenatal diagnosis
 3-hydroxy-3-methylglutaryl CoA lyase deficiency 330
 adenosine deaminase deficiency 509
 adrenoleukodystrophy 464
 α 1-antitrypsin (AT) deficiency 807
 argininosuccinic aciduria 219
 biotinidase deficiency 52
 Canavan disease 815–16
 carnitine palmitoyl transferase II deficiency, lethal neonatal 273
 carnitine–acylcarnitine translocase deficiency 264
 citrullinemia 212, 214
 cystinosis 535
 familial hypercholesterolemia 636
 fucosidosis 742
 galactosemia 419
 galactosialidosis 756
 glutaric aciduria type I 71
 GM₁ gangliosidosis 672
 holocarboxylase synthetase deficiency 43
 Hunter disease 555, 576
 Hurler disease 555
 4-hydroxybutyric aciduria 92
 D-2-hydroxyglutaric aciduria 81
 hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin 128
 I-cell disease 618
 isovaleric acidemia 59
 Krabbe disease 729
 Lesch–Nyhan disease 491
 α -mannosidosis 748
 Menkes disease 549
 3-methylcrotonyl CoA carboxylase deficiency 76
 methylmalonic acidemia 24–5
 mevalonic aciduria 645
 mitochondrial acetoacetyl-CoA thiolase deficiency 98
 mucopolipidosis III 626
 multiple acyl CoA dehydrogenase deficiency 320
 NARP 391
 neonatal adrenoleukodystrophy 474
 Niemann–Pick disease 713, 715
 Niemann–Pick type C disease 722
 nonketotic hyperglycinemia 184
 organic acidemias 5
 ornithine transcarbamylase deficiency 199, 200–1
 Pearson syndrome 402
 Pompe disease 443–4
 propionic acidemia 13–14
 pyruvate carboxylase deficiency 351
 Sandhoff disease 691
 Sanfilippo disease 585
 short/branched chain acyl CoA dehydrogenase deficiency 314
 Tay–Sachs disease 683
 Wolman disease 737
 Zellweger syndrome 474
 prenatal therapy, holocarboxylase synthetase (HCS) deficiency 44
 PREPL gene 528
 primapertine 125
 progressive external ophthalmoplegia (PEO), Kearns–Sayre syndrome 393, 394
 prolactin levels
 aromatic L-amino acid decarboxylase deficiency 138
 hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin 131
 tyrosine hydroxylase deficiency 140
 proline
 lysineric protein intolerance 237
 multiple acyl CoA dehydrogenase deficiency 320
 pyruvate carboxylase deficiency 348
 propionic acid 14
 metabolism **8**, **8**
 methylmalonic acidemia 25, 27
 propionic acidemia 8–16, 98, 338
 clinical abnormalities **9**, 9–13, **10**, **11**, **12**, **13**
 genetics and pathogenesis 13–14
 treatment 14–16, 15
 propionyl CoA carboxylase **8**, **8**
 activity in propionic acidemia 13, 13
 prostate stones, alkaptonuria 108
 protective protein/cathepsin A *see* PPCA (protective protein/cathepsin A)
 protein elevation, cerebrospinal fluid *see* cerebrospinal fluid (CSF) protein elevation
 protein inadequacy, citrullinemia 214, **214**
 protein intolerance *see* dietary protein intolerance
 proteinuria
 cobalamin C disease 36
 congenital disorders of glycosylation, type Ia 784
 cystinosis 533
 Fabry disease 661
 galactosialidosis 754
 PRPS1 gene 503, 504–5
 PRPS2 gene 503, 504–5
 pruritus, α 1-antitrypsin (AT) deficiency 804, 807
 pseudochyloous ascites, α 1-antitrypsin (AT) deficiency 804
 pseudo-Hurler polydystrophy *see* mucopolipidosis III
 pseudo-neonatal adrenoleukodystrophy 472
 pseudotumor cerebri, galactosemia **417**, 418

- psychiatric abnormalities 844
 Hartnup disease 541
 homocystinuria 147
 metachromatic leukodystrophy 762
- psychomotor impairment
 argininemia 223
 CDG type Ic 789
 CDG type Id 790
 CDG type Ie 791
 CDG type If 792
 CDG type IIg 795
 galactosialidosis 753
 4-hydroxybutyric aciduria 89
 L-2-hydroxyglutaric aciduria 85, 87
 I-cell disease 614–15
 Krabbe disease 727
 mevalonic aciduria 643
- psychosis, Hurler–Scheie disease 568
- psychotic behavior 844
 adult GM₂ gangliosidosis 681
 Sanfilippo disease 583
see also behavior problems
- pterin-4 α -carbinolamine dehydratase (PCD) 123, 124
- pterin-4 α -carbinolamine dehydratase (PCD) deficiency 125, 128, 129, 130
- ptosis 844
 aromatic L-amino acid decarboxylase deficiency 137
 Kearns–Sayre syndrome 393, 393, 394
 lactic acidemias 344
 MELAS 377
 tyrosine hydroxylase deficiency 139
- pulmonary disease
 acute neuronopathic Gaucher disease 702
 adenosine deaminase deficiency 508
 α 1-antitrypsin (AT) deficiency 804, 805
 Gaucher disease 701
 lysinuric protein intolerance 237, 238
 Niemann–Pick disease 709–10, 711
 Niemann–Pick type C disease 720
see also specific pulmonary diseases
- purine metabolism disorders 483–520
 adenine phosphoribosyl-transferase deficiency *see* adenine phosphoribosyl-transferase (APRT) deficiency
 adenosine deaminase deficiency *see* adenosine deaminase (ADA) deficiency
 adenylosuccinate lyase deficiency *see* adenylosuccinate lyase (ASL) deficiency
 Lesch–Nyhan disease *see* Lesch–Nyhan disease
 orotic aciduria *see* orotic aciduria
 phosphoribosylpyrophosphate synthetase abnormalities 503–5, 504
- PXR1 (peroxisomal assembly protein) 470
- pyloric stenosis 844
 D-2-hydroxyglutaric aciduria 79
 phenylketonuria 113
- pyramidal tract signs, propionic acidemia 10
- pyridox(am)ine 5'-phosphate oxidase (PNPO) deficiency 138, 138
- pyridoxine therapy
 aromatic L-amino acid decarboxylase deficiency 139
 homocystinuria 147, 148, 149
- pyroglutamic aciduria 338
- pyruvate carboxylase 347, 347–8, 351
- pyruvate carboxylase deficiency 347–52
 clinical abnormalities 348, 348–51, 350
 genetics and pathogenesis 347, 351
 treatment 352
- pyruvate dehydrogenase complex (PDHC) 359, 360, 368
- pyruvate dehydrogenase complex (PDHC) deficiency 340, 359–64, 368
 clinical abnormalities 360–2, 361, 369
 genetics and pathogenesis 362–3, 371
 treatment 364, 372
- pyruvate metabolism 337, 337, 338
 disorders *see* lactic acidemia(s)
- 6-pyruvoyltetrahydropterin synthase (6-PTS) 124, 124, 125
- 6-pyruvoyltetrahydropterin synthase (6-PTS) deficiency 125, 125, 126, 127, 128, 128–9
 treatment 131
- quadripareisis/quadriplegia *see* spastic quadripareisis/quadriplegia
- ragged red fibers 339, 340, 341, 344, 844
 Kearns–Sayre syndrome 393, 394
 MELAS 377, 377
 MERRF disease 383
 mitochondrial DNA depletion 404, 407
- Rambam–Hasharon syndrome 794
- rBAT protein 528
- red urine 844
- reducing substance, urinary, galactosemia 418, 421
- Reilly bodies 562, 563
- renal calculi 844
 adenine phosphoribosyl-transferase deficiency 499
 cystinuria 526, 526, 526–7
 hypoxanthine–guanine phosphoribosyl transferase deficiency 488
see also urinary tract calculi
- renal cysts 844
- renal disease
 Fabry disease 661
 hepatorenal tyrosinemia 173–4, 175–6
 lysinuric protein intolerance 237, 238
 multiple acyl CoA dehydrogenase deficiency 317, 319
 von Gierke disease 431, 432, 434
see also polycystic kidneys; *specific renal diseases*
- renal failure
 adenine phosphoribosyl-transferase deficiency 499
 cystinosis 533–4
 Fabry disease 661, 662
 Lesch–Nyhan disease 487–8
 lysinuric protein intolerance 237
- renal Fanconi syndrome *see* Fanconi syndrome
- renal impairment, methylmalonic acidemia 23–4
- renal transplantation
 Fabry disease 662
 methylmalonic acidemia 28
 nephropathic cystinosis 536
- renal tubular acidosis (RTA) 845
 carnitine palmitoyl transferase I deficiency 269
 glycogenosis type III 450
- Kearns–Sayre syndrome 394
- lactic acidemia 370, 372
- MELAS 377
- pyruvate carboxylase deficiency 350, 352
- respiratory alkalosis
 argininosuccinic aciduria 216
 carbamyl phosphate synthetase deficiency 206
 ornithine transcarbamylase deficiency 198
- respiratory infections
 Hunter disease 575
 Hurler disease 558
 I-cell disease 616
 Niemann–Pick disease 709
 Sly disease 606
- respiratory insufficiency, lysinuric protein intolerance 237
- retinal degeneration
 cobalamin C disease 34
 congenital disorders of glycosylation, type Ia 783
- retinal detachment, homocystinuria 145
- retinal discoloration, metachromatic leukodystrophy 761
- retinal disease, ichthyosis and 841
- retinal hemorrhages, glutaric aciduria type I 68
- retinal lesions, von Gierke disease 432
- retinal vessels, ethylmalonic encephalopathy 821, 822
- retinitis, multiple sulfatase deficiency 773
- retinitis pigmentosa 845
 congenital disorders of glycosylation, type Ia 783
 Hunter disease 575
 Kearns–Sayre syndrome 393, 394
 mevalonic aciduria 644
 NARP 388, 389, 390
 neonatal adrenoleukodystrophy 472, 473, 474
 Sandhoff disease 688
- retinopathy
 cobalamin C disease 34
 cystinosis 534
 long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 297, 299
- Reye syndrome presentation 845
 carnitine palmitoyl transferase I deficiency 269
 carnitine transporter deficiency 254, 255
 fatty acid oxidation disorders 247
 glutaric aciduria type I 66
 hepatorenal tyrosinemia 173
 HHH syndrome 230, 231
 3-hydroxy-3-methylglutaryl CoA lyase deficiency 326, 329
 hyperammonemia 198, 199
 isovaleric acidemia 58
 long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 296, 297
 medium-chain acyl CoA dehydrogenase deficiency 281, 283
 3-methylcrotonyl CoA carboxylase deficiency 75
 mitochondrial DNA depletion 405
 very long-chain acyl CoA dehydrogenase deficiency 290
- Reynaud syndrome 845

- rhabdomyolysis 845
 carnitine palmitoyl transferase II deficiency, late onset 278
 glutaric aciduria type I 67
 long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 296, 297
 very long-chain acyl CoA dehydrogenase deficiency 290
 riboflavin therapy
 glutaric acidemia I 71–2
 medium-chain acyl CoA dehydrogenase deficiency 287
 MELAS 379
 multiple acyl CoA dehydrogenase deficiency 321
 riboflavin transporter defect 322
 Richner–Hanhart syndrome 164
 rickets
 cystinosis 532, 533, 536
 hepatorenal tyrosinemia 171, 174
 rigidity
 hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin 125, 129
 Krabbe disease 727
 late infantile/juvenile GM1 gangliosidosis 669
 tyrosine hydroxylase deficiency 139
 tRNA lysine, mutations 382, **382**, 384
 roentgenograms
 adenosine deaminase deficiency 508, 509
 alkaptonuria 107, 108, **108**
 α 1-antitrypsin (AT) deficiency **804**, 805
 cystinosis 533
 cystinuria 526, **526**
 fucosidosis 741
 Gaucher disease 700–1, **701**
 GM₁ gangliosidosis 668, **668**, **669**
 homocystinuria 146, **147**
 Hunter disease **574**, **575**, 576
 Hurler disease 557, 560–1, **561**, **562**
 hypoxanthine–guanine phosphoribosyl transferase deficiency **487**
 I-cell disease **616**, 617, **617**
 Lesch–Nyhan disease **486**
 lysinuric protein intolerance 237
 α -mannosidosis 746, **747**
 Maroteaux–Lamy disease **600**, 601, **601**
 Menkes disease 548, 549
 Morquio syndrome 591, **592**, **593**
 mucopolipidosis III **621**, 621–2, **622**, **623**
 multiple sulfatase deficiency 771, 773, **774**
 Pompe disease 439, **440**
 Sanfilippo disease 583–4, **584**
 Sly disease 607
 Wolman disease 734, 735

 SAH (S-adenosylhomocysteine) hydrolase 510
 Sandhoff disease 678, 679, 681, 686–91
 clinical abnormalities **686**, 686–9, **687**, **688**, **689**
 genetics and pathogenesis **679**, 689–91
 treatment 691
 Sanfilippo disease 556, 580–6
 clinical abnormalities 580–4, **581**, **582**, **583**, **584**
 genetics and pathogenesis **581**, 585
 treatment 585–6

 saposin B 760, 763
 saposin B deficiency, metachromatic leukodystrophy 761, 762, 763, 764
 sapropterin dihydrochloride 118, 130
 Saudi variant, Niemann–Pick disease 711
 SBCAD (short/branched chain acyl CoA dehydrogenase) deficiency 312–14, **313**
 SCAD (short-chain acyl CoA dehydrogenase) 281, 302, **302**
 SCAD deficiency *see* short-chain acyl CoA dehydrogenase (SCAD) deficiency
 SCHAD (short-chain 3-hydroxyacyl CoA dehydrogenase) 309, **309**, 310
 SCHAD deficiency **309**, 309–10
 Scheie disease 556, 566–70
 clinical abnormalities **566**, 567
 genetics and pathogenesis 569–70
 treatment 570
 scoliosis 845
 Morquio syndrome 591
 Pompe disease 441
 see also kyphoscoliosis
 SCOT (succinyl coA:3-oxoacid CoA transferase) deficiency 100
 sea-blue histiocytes
 Niemann–Pick disease 710, 711–12
 Niemann–Pick type C disease 720
 seborrheic dermatitis, holocarboxylase synthetase deficiency 41
 Segawa disease 125, 128
 seizures
 adenylosuccinate lyase deficiency 515, 516
 adrenoleukodystrophy 460, 461
 arginine:glycine amidinotransferase deficiency 828
 biotinidase deficiency 48, 50–1
 Canavan disease 813, 816
 CDG type If 791, 792
 CDG type Ii 793
 CDG type Ij 793
 CDG type Ik 793
 CDG type IL 793
 CDG type In 793
 CDG type IIb 794
 CDG type IIc 794
 CDG type IIe 794
 CDG type IIh 795
 CDG type IIj 795
 citrullinemia 211, 212
 cobalamin C disease 34, 35
 congenital disorders of glycosylation, type Ia 784
 creatine transporter deficiency 828
 dihydrofolate reductase deficiency 141
 ethylmalonic encephalopathy 820
 Fabry disease 661
 galactosialidosis 755
 Gaucher disease, type III 702
 glutamine synthetase deficiency 241, 242, 243
 guanidinoacetate methyltransferase deficiency 828, 829
 hepatorenal tyrosinemia 174
 HHH syndrome 230
 homocystinuria 147
 3-hydroxy-3-methylglutaryl CoA lyase deficiency 326
 4-hydroxybutyric aciduria 89, 91, 92

 D-2-hydroxyglutaric aciduria 80
 L-2-hydroxyglutaric aciduria 86, 87
 hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin 125, 126
 Krabbe disease 728
 lactic acidemias 343
 late infantile/juvenile GM1 gangliosidosis 669
 Lesch–Nyhan disease 485
 medium-chain acyl CoA dehydrogenase deficiency 282, 283
 MELAS 376, 378
 Menkes disease 547
 MERRF disease 383
 metachromatic leukodystrophy 761, 762
 3-methylcrotonyl CoA carboxylase deficiency 75
 mitochondrial acetoacetyl-CoA thiolase deficiency 96
 NARP 388, 389, 390, 391
 neonatal adrenoleukodystrophy 470
 Niemann–Pick type C disease 719–20, 722
 nonketotic hyperglycinemia 181, 182, 185
 phenylketonuria 115
 propionic acidemia 10
 pyruvate carboxylase deficiency 350
 pyruvate dehydrogenase complex deficiency 361
 Sandhoff disease 687
 Tay–Sachs disease 680
 very long-chain acyl CoA dehydrogenase deficiency 290
 see also convulsions
 self-injurious behavior 845
 Lesch–Nyhan disease **485**, 485–6, **486**, 494
 propionic acidemia 10, **11**
 sensorineural deafness 845
 MELAS 377
 Morquio syndrome 591
 see also deafness
 sepiapterin reductase 124
 deficiency 128
 sepsis neonatorum, galactosemia 417
 sepsis, propionic acidemia 9
 serine, lysinuric protein intolerance 237
 serum glutamate pyruvate transaminase (SGPT) 212
 serum glutamate-oxaloacetate transaminase (SGOT) 211–12
 serum transferrin, congenital disorders of glycosylation, type Ia 781, 784–5
 severe combined immunodeficiency disease (SCID), adenosine deaminase deficiency 507–8, 509, 510
 short stature
 cystinosis 534
 fucosidosis 741
 galactosialidosis 754
 Hurler disease 560
 I-cell disease 615
 Maroteaux–Lamy disease 597, **598**
 Morquio syndrome 589, **589**, 591
 multiple sulfatase deficiency 773, **773**
 Pearson syndrome 401
 phenylketonuria 115
 Sly disease 606
 von Gierke disease 429, 434

- short/branched chain acyl CoA dehydrogenase (SBCAD) deficiency 312–14, **313**
- short-chain 3-hydroxyacyl CoA dehydrogenase (SCHAD) 309, **309**, 310
- short-chain 3-hydroxyacyl CoA dehydrogenase (SCHAD) deficiency **309**, 309–10
- short-chain acyl CoA dehydrogenase (SCAD) 281, 302, **302**
- short-chain acyl CoA dehydrogenase (SCAD) deficiency **249**, 250, 302–6, 820, 824
- clinical abnormalities **304**, 304–5, **305**
- genetics and pathogenesis **302**, 305–6
- treatment 306
- Shwachman syndrome 401
- sideroblastic anemia, lactic acidemias 344
- skeletal abnormalities
- adenosine deaminase deficiency 508–9
 - congenital disorders of glycosylation, type Ia 784
 - fucosidosis 741
 - Gaucher disease 699–700, **701**
 - GM₁ gangliosidosis 668, **668**, **669**
 - α -mannosidosis 745–6, **746**
 - Morquio B disease 671
 - Morquio syndrome 589, **589**, **590**, 591, **591**, 593
 - Sly disease 606, 607
 - see also specific abnormalities*
- skin, homocystinuria 146
- skin lesions
- biotinidase deficiency **48**, **49**, 49–50, **50**, 54
 - cobalamin C disease 34–5
 - ethylmalonic encephalopathy **820**, 821, **821**
 - Fabry disease 660, **660**
 - glutamine synthetase deficiency 241–2, **242**
 - holocarboxylase synthetase deficiency 41
 - Hunter disease 573, **573**
 - lysine protein intolerance 236, **236**
 - methylmalonic acidemia 21, **21**
 - oculocutaneous tyrosinemia 165, 166, **166**
 - phenylketonuria 113, **113**, 114
 - see also specific skin lesions*
- skin pigmentation
- adrenoleukodystrophy 459, 461
 - alkaptonuria 107, **107**
 - Gaucher disease 701
 - homocystinuria 145
 - phenylketonuria 114, **114**
- SLC3A1 gene 528
- Sly disease 556, 605–8
- clinical abnormalities **606**, 606–7, **607**
 - genetics and pathogenesis **605**, 607–8
 - treatment 608
- small bowel changes, Wolman disease 736
- Smith–Magenis syndrome 486
- smoking, α 1-antitrypsin (AT) deficiency 805, 807
- sodium benzoate *see* benzoate therapy
- somnolence, 4-hydroxybutyric aciduria 92, 93
- Sotos syndrome 21
- spastic paraparesis/paraplegia 845–6
- biotinidase deficiency 51, **51**
 - HHH syndrome 230, 231
 - α -mannosidosis 746
 - Maroteaux–Lamy disease 600
 - MERRF disease 383
- spastic quadriplegia/quadruplegia
- adrenoleukodystrophy 460
 - argininemia 223
 - carbamyl phosphate synthetase deficiency 206
 - metachromatic leukodystrophy 761, 762
 - multiple sulfatase deficiency 770, 773, 776
 - NARP 390
 - propionic acidemia 10
- spasticity
- argininemia 223, **224**
 - Canavan disease 812–13, **813**
 - glutaric aciduria type I 64, 65, **67**
 - GM₁ gangliosidosis, adult 670
 - GM₁ gangliosidosis, infantile 668
 - GM₁ gangliosidosis, late infantile/juvenile 669
 - Hurler–Scheie disease 568
 - D-2-hydroxyglutaric aciduria 80
 - Krabbe disease 727, **728**
 - Lesch–Nyhan disease 484, 494
 - MERRF disease 383
 - phenylketonuria 115
 - Sandhoff disease 687, 688
 - Tay–Sachs disease 681
- speech problems 841
- arginine:glycine amidinotransferase deficiency 828, 830
 - aromatic L-amino acid decarboxylase deficiency 137
 - creatine transporter deficiency 828
 - galactosemia 418
 - guanidinoacetate methyltransferase deficiency 829
 - 4-hydroxybutyric aciduria 89, 91
 - D-2-hydroxyglutaric aciduria 79
 - L-2-hydroxyglutaric aciduria 86
 - Lesch–Nyhan disease 485
 - mitochondrial acetoacetyl-CoA thiolase deficiency 96
 - see also dysarthria*
- sphingomyelin **708**, 713–14
- sphingomyelinase 709, **709**, 712–13
- sphingomyelinase deficiency 708, 711, 713–14
- see also* Niemann–Pick type A disease; Niemann–Pick type B disease
- spider telangiectases, α 1-antitrypsin (AT) deficiency 804
- spinal cord compression, Morquio syndrome 593
- splenectomy
- Gaucher disease 700, 704–5
 - Niemann–Pick disease 710
- splenomegaly
- acute neuronopathic Gaucher disease 702
 - cholesteryl ester storage disease 736
 - galactosemia 415
 - Gaucher disease 698, 699
 - Maroteaux–Lamy disease 599
 - Wolman disease 734, 736
 - see also* hepatosplenomegaly
- spondyloepiphyseal metaphyseal dysplasia, fucosidosis 741
- spongy degeneration, Canavan disease 814, **814**
- spontaneous pneumothorax, homocystinuria 148
- statin therapy
- cholesterol ester storage disease 737
 - heterozygous familial hypercholesterolemia 637
- stature, short *see* short stature
- steatorrhea, Pearson syndrome 399
- strabismus, L-2-hydroxyglutaric aciduria 87
- stridor
- biotinidase deficiency 51
 - D-2-hydroxyglutaric aciduria 80
- stroke-like episodes 548, 846
- carbamyl phosphate synthetase deficiency 206
 - cobalamin C disease 35
 - congenital disorders of glycosylation, type Ia 782, 783, 784
 - Fabry disease 661
 - homocystinuria 147, 148
 - MELAS 374, 375, 376, 378, 379
 - 3-methylcrotonyl CoA carboxylase deficiency 75
 - propionic acidemia 11
- subacute necrotizing encephalomyelopathy, lactic acidemia 343, **343**
- subdural effusions 846
- glutaric aciduria type I 68
 - D-2-hydroxyglutaric aciduria 79, 80
 - pyruvate carboxylase deficiency 350
- subdural hematomas, glutaric aciduria type I 68, **69**
- subependymal cysts
- glutamine synthetase deficiency 242, **243**
 - holocarboxylase synthetase deficiency 42
- substrate deprivation therapy, Sandhoff disease 691
- substrate reduction therapy, Gaucher disease 704
- succinic semialdehyde dehydrogenase **90**, 92
- deficiency *see* 4-hydroxybutyric aciduria
- succinyl coA:3-oxoacid CoA transferase (SCOT) deficiency 100
- succinylacetoacetone, hepatorenal tyrosinemia 171, **172**, 174, 175, 176
- succinyladenosine levels, adenosine deaminase deficiency 515
- succinylaminoimidazolecarboxamide ribotide (SAICAR) levels, adenosine deaminase deficiency 514, 515
- sudden infant death syndrome (SIDS) 247
- carnitine transporter deficiency 255
 - carnitine–acylcarnitine translocase deficiency 261
 - 3-hydroxyacyl CoA dehydrogenase deficiency 309
 - medium-chain acyl CoA dehydrogenase deficiency 282, 283, 286
- sulfatase enzymes 769, 769
- activation 769, **769**
 - see also specific sulfatases*
- sulfatides, metachromatic leukodystrophy 760, 763, 764
- SUMF1 gene 769–70
- surgical interventions
- Hunter disease 577
 - Hurler disease 564
 - Hurler–Scheie disease 570
 - Maroteaux–Lamy disease 602
 - Morquio syndrome 594
 - mucopolidosis III 626
 - see also* joint replacement

- swallowing difficulties *see* dysphagia
- sweating
fucosidosis 741
glutaric aciduria type I 66
- tachycardia, fructose-1,6-diphosphatase deficiency 355
- tachypnea
argininemia 224
argininosuccinic aciduria 216
holocarboxylase synthetase deficiency 41
multiple acyl CoA dehydrogenase deficiency 317
- talipes equinovarus 607, 614
- tandem mass spectrometry (MS/MS) 3
glutaric aciduria type I diagnosis 70
isovaleric acidemia diagnosis 61
maple syrup urine disease 158
3-methylcrotonyl CoA carboxylase deficiency diagnosis 77
methylmalonic acidemia diagnosis 24, 25–6
see also acylcarnitine profiles
- Tarui disease 426, 427
- Tay–Sachs disease 678–83, 679
clinical abnormalities 678–81, **680, 681**
genetics and pathogenesis **679**, 681–3, 682, 683
treatment 683
- teeth
colored 846
Hurler disease 559
Maroteaux–Lamy disease 601
Morquio syndrome 591
mucopolidosis III 624, **625**
- telangiectases
 α 1-antitrypsin (AT) deficiency 804
Fabry disease **660**
galactosialidosis 754
- tetrahydrobiopterin (BH_4) 123, **123**
cerebral depletion in dihydrofolate reductase deficiency 141
- tetrahydrobiopterin (BH_4) metabolism **123**, 124, **124**
disorders 124, 125, 128
see also hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin
- tetrahydrobiopterin (BH_4) therapy 130, **130**
- thiamine therapy
maple syrup urine disease 158, 159
pyruvate dehydrogenase complex deficiency 364
- thiamine-responsive branched-chain oxoaciduria 156, 159
- threonine intolerance, methylmalonic acidemia 20
- thrombocytopenia
galactosialidosis 754
Gaucher disease 699
isovaleric acidemia 59
methylmalonic acidemia 24
Niemann–Pick disease 710, 711
propionic acidemia 11
- thrombosis
CDG type Ib 789
homocystinuria 146–7, 148, 149
- thymus, adenosine deaminase deficiency 508, 509
- thyroxine-binding globulin (TBG), congenital disorders of glycosylation, type Ia 781, 784
- tiglylglycine
holocarboxylase synthetase deficiency 42
mitochondrial acetoacetyl-CoA thiolase deficiency 98, **98**
- transaminases
argininemia 225
arginosuccinic aciduria 218
carnitine transporter deficiency 254
carnitine–acylcarnitine translocase deficiency 263
CDG type Ib 789
citrullinemia 212
congenital disorders of glycosylation, type Ia 782
glycogenosis type III 450, 453
long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 296, 297
3-methylcrotonyl CoA carboxylase deficiency 75
see also alanine aminotransferase (ALT); aspartate aminotransferase (AST)
- transferrin, congenital disorders of glycosylation, type Ia 781, 784–5
- transient hyperammonemia of the newborn 191, 194
- transient ischemic attacks, Fabry disease 661
- transient phenylalaninemia 118
- transport disorders
cystinosis *see* cystinosis
cystinuria *see* cystinuria
Hartnup disease **540**, 540–2, **541, 542**
histidinuria **544**, 544–5, **545**
Menkes disease *see* Menkes disease
- tremors
citrullinemia 212
hyperphenylalaninemia and defective metabolism of tetrahydrobiopterin 126, 129
isovaleric acidemia 59
Niemann–Pick type C disease 718, 722
- trichorrhexis nodosa
arginosuccinic aciduria 42, 217, **217**
Menkes disease 547, 547
- trifunctional protein (TFP) 295
deficiency 295, 296, 297, 298
- triglycerides
apolipoprotein C-II deficiency 653
familial hypercholesterolemia 634, 650
lipoprotein lipase deficiency 648, 650, 650, 651–2, 653
- trihexyphenidyl therapy, aromatic L-amino acid decarboxylase deficiency 139
- tRNA lysine, mutations 382, **382**, 384
- tryptophan malabsorption, Hartnup disease 540
- tryptophanuria, Hartnup disease 541
- tuberous xanthomas, familial hypercholesterolemia 632
- tubulointerstitial nephritis, methylmalonic acidemia 24
- tumors, brain, L-2-hydroxyglutaric aciduria 87
- Turkish mustache sign 687, **689**
- type 1 hyperlipoproteinemia *see* lipoprotein lipase deficiency
- tyrosine aminotransferase **164**, 165, 167
deficiency *see* oculocutaneous tyrosinemia
- tyrosine hydroxylase **137**, 140
deficiency 138, 139–40
- tyrosine metabolism **164**, **172**
- tyrosinemia
hepatorenal *see* hepatorenal tyrosinemia
hyperphenylalaninemia and 117
oculocutaneous *see* oculocutaneous tyrosinemia
- tyrosinemia type I *see* hepatorenal tyrosinemia
- tyrosinemia type II 164
- tyrosinosis *see* hepatorenal tyrosinemia
- ubiquinone concentrations, mevalonic aciduria 646
- upward gaze, paralysis *see* paralysis of upward gaze
- urea cycle 191, **191**
- urea cycle disorders 191–6
argininemia *see* argininemia
argininosuccinic aciduria *see* argininosuccinic aciduria
carbamyl phosphate synthetase deficiency *see* carbamyl phosphate synthetase (CPS) deficiency
citrullinemia *see* citrullinemia
glutamine synthetase deficiency *see* glutamine synthetase deficiency
HHH syndrome *see* HHH (hyperornithinemia, hyperammonemia, homocitrullinuria) syndrome
lysine protein intolerance *see* lysinuric protein intolerance
ornithine transcarbamylase deficiency *see* ornithine transcarbamylase (OTC) deficiency
see also hyperammonemia
- 3-ureidopropionate 14
- uric acid levels
carnitine transporter deficiency 254
Lesch–Nyhan disease 486–7
long-chain L-3-hydroxyacyl CoA dehydrogenase deficiency 296, 297
medium-chain acyl CoA dehydrogenase deficiency 283
phosphoribosylpyrophosphate synthetase abnormalities 504
see also hyperuricemia
- uridine therapy, orotic aciduria 520
- uridine-5-monophosphate (UMP) synthase 520
- urinary reducing substance, galactosemia 418, 421
- urinary tract calculi
adenine phosphoribosyl-transferase deficiency 499
alkaptonuria 108
cystinosis 533
cystinuria **526**, 526–7, 528, 529
hypoxanthine–guanine phosphoribosyl transferase deficiency 484, 487, **487**, 488, 494
see also renal calculi
- urinary tract infections
cystinuria 526
D-lactic aciduria 7
- urine
alkaptonuria 105–6, **106**

- urine – *cont.*
 maple syrup urine disease 154, 155, **155**, 157, 157
 organic acid analysis 5, 6, 6
 red 844
 uveitis, mevalonic aciduria 644
- vacuolated granulocytes, Wolman disease 735, **735**
- vacuolated lymphocytes
 fucosidosis 741
 galactosialidosis 755
 GM₁ gangliosidosis 669
 I-cell disease 617
 α -mannosidosis 747, **747**
 Wolman disease 735
- vacuoles
 fucosidosis 741–2
 galactosialidosis 755
 GM₁ gangliosidosis 668–9
 α -mannosidosis 747–8
 Wolman disease 735, **735**
- valine intolerance, methylmalonic acidemia 20
- valine metabolism **152**
- valproic acid 7
 glutaric acidemia I management 72
- vascular disease
 homocystinuria 146–7, **147**, 148
see also cerebral vascular disease
- ventricular hemorrhage, propionic acidemia 9
- vertebra plana, Morquio disease 591
- very long-chain acyl CoA dehydrogenase (VLCAD) 281, 289, **289**, 291
- very long-chain acyl CoA dehydrogenase (VLCAD) deficiency **249**, 250, 289–92
 clinical abnormalities 289–91, **290**, **291**
 genetics and pathogenesis **289**, 291–2
 treatment 292
- very long-chain acyl CoA synthetase (VLCAS) **459**, 460, 464
- very long-chain fatty acids (VLCFA) 459, 463
 accumulation in adrenoleukodystrophy 459, 463, 464
 accumulation in neonatal
 adrenoleukodystrophy 469, 474, 476
 peroxisomal β -oxidation **473**
- very low density lipoproteins (VLDL) levels, inherited hyperlipidemias 650
- vigabatrin therapy
 glutaric acidemia I 72
 4-hydroxybutyric aciduria 93
- vision loss
 adrenoleukodystrophy 460
see also blindness
- visual evoked potentials (VEP), biotinidase deficiency 52
- visual evoked response (VER), acute neuronopathic Gaucher disease 702
- visual hallucinations 846
- visual impairment
 adrenoleukodystrophy 460
 biotinidase deficiency 51
 cobalamin C disease 34, 35
 cystinosis 534
 Fabry disease 661
 NARP 388
 Scheie disease 567
see also blindness
- vitamin D receptor abnormalities, alopecia 42
- VLCAD (very long-chain acyl CoA dehydrogenase) 281, 289, **289**, 291
 deficiency *see* very long-chain acyl CoA dehydrogenase (VLCAD) deficiency
- voice
 Hunter disease 574
 I-cell disease 615
- vomiting 844
 3-hydroxy-3-methylglutaryl CoA lyase deficiency 326, 329
 α 1-antitrypsin (AT) deficiency 804
 argininemia 223
 argininosuccinic aciduria 216, 217, 220
 carbamyl phosphate synthetase deficiency 206
 CDG type Ib 789
 CDG type If 791
 CDG type Ih 793
 citrullinemia 210, 211, 212, 214
 ethylmalonic–adipic aciduria 319
 fructose-1,6-diphosphatase deficiency 355, 357
 galactosemia 415
 glutaric aciduria type I 65, 71
 hepatorenal tyrosinemia 172
 HHH syndrome 230
 holocarboxylase synthetase deficiency 41, 42
- D-2-hydroxyglutaric aciduria 79
 isovaleric acidemia 58
 Krabbe disease 728
 lactic acidemias 343
 lysinuric protein intolerance 236
 maple syrup urine disease 153, 156
 medium-chain acyl CoA dehydrogenase deficiency 282, 283, 286
 3-methylcrotonyl CoA carboxylase deficiency 75
 methylmalonic acidemia 20, 28
 mevalonic aciduria 644
 mitochondrial acetoacetyl-CoA thiolase deficiency 96, 98
 mitochondrial DNA depletion 405, 406, 407
 nonketotic hyperglycinemia 181
 ornithine transcarbamylase deficiency 199
 phenylketonuria 113
 Wolman disease 733
- von Gierke disease 425, 426, 426, 428–34
 clinical abnormalities **429**, 429–32, **430**, **432**
 genetics and pathogenesis 432–3
 glycogenosis type III vs. 451, 451
 treatment 433–4
- white matter
 Canavan disease 814
see also cerebellum
- Wolff–Parkinson–White syndrome 377
- Wolman disease 432, 733–7
 clinical abnormalities 733–6, **734**, **735**, **736**
 genetics and pathogenesis 733, **733**, 737
 treatment 737
- xanthelasma, familial
 hypercholesterolemia 634
- xanthomas 846
 familial hypercholesterolemia 632, 633, **633**, 634, **634**, 636
see also cutaneous xanthomas
- X-linked cutis laxa 549
- zebra bodies 562, 584, 695, **762**
- Zellweger syndrome 469, 470
 clinical abnormalities **471**, 471–2, **472**
 genetics and pathogenesis 474, 475, 476
 treatment 476